STUDIES IN THE PHYSIOLOGY OF THE FUNGI

II. Lenzites saepiaria Fries, with Special Reference to Enzyme Activity

SANFORD M. ZELLER
Research Fellow in the Henry Shaw School of Botany of Washington University[1]

Introduction

This paper reports the results of an experimental study relating to certain physiological activities of the wood-destroying fungus, *Lenzites saepiaria*. The investigation here reported is concerned primarily with cultural characteristics, some of the factors influencing growth and metabolism, and the enzymic activity in the fungus. Special attention is given to the cyto-hydrolyzing enzymes and the relation of these to the decay produced by *Lenzites*.[2]

The Fungus

This fungus is commonly known by lumbermen as the "brown punk" because of the sepia color of the small bracket-like sporophores. In nature it is generally found on railroad ties, telephone and telegraph poles, and less often on standing timber. It attacks coniferous timber, as a rule, but is known to attack frondose wood (Weir, '14). The sporophores appear near cracks in the wood due to drying. The fruiting surface is made up of branching gills which may become so much anastomosed that the pores formed are very much daedaloid. The mycelium is best studied in pure cultures.

---

[1] A fellowship established by the Southern Pine Association, New Orleans, La.

[2] In the fall of 1914, in coöperation with the Southern Pine Association of New Orleans, it was decided to attempt a determination of the natural factors of wood influencing in any marked degree the growth and destructive properties of wood-destroying fungi, that is, durability with respect to fungous decay. While the results in this limited field of investigation will be reported in a later paper, the present study deals with those additional physiological phases which were a necessary and fundamental part of the general plan.

While in search for a fungus suitable to employ in such a problem, it was deemed important to make the results as far-reaching in the economic world as possible. Since practically three-fourths of the structural timber used in the United States is furnished by the coniferous species of trees, and since *Lenzites saepiaria* Fries is considered the fungus most destructive to coniferous wood, this fungus was used in the investigation.

CULTURE RELATIONS

Two methods of securing pure cultures were employed. They may be designated as (1) the tissue method, and (2) the spore method.

(1) The tissue method was first described by Duggar ('05), who applied this method to the making of spawn in mushroom culture. In connection with this study on *Agaricus campestris* tests were made with 69 species of basidiomycetous fungi on various media. Forty of these grew promptly on the media employed. The method was suggested by the fact that "during moist weather, or in a moist cellar where mushrooms are being grown, one will frequently find that an injury in a young mushroom is rapidly healed by a growth of hyphae from the edges of the injured area. The same thing had been noticed in the open in the case of puffballs. In many instances, moreover, pure cultures of fungi in other groups have been obtained by small bits of a sclerotial mass of tissue." Accordingly, young sporophores were obtained, "and after breaking them open longitudinally a number of pieces of tissue from within were carefully removed with a sterile scalpel to a sterile Petri dish." A number of cultures were then made by transferring these nocules of tissue to various forms of nutrient media, such as bean pods, manure, leaf mould, etc. From this and from numerous other similar tests it was ascertained that when the sporophores from which the nocules of tissue were taken were young and healthy, there was seldom an instance in which growth did not result. It was shown that failure to grow was generally due to the advanced age of the sporophore used, to an unfavorable medium, or to bacterial contamination.

In my work dried sporophores were used for the tissue method. These were collected in a freshly growing condition and dried at room temperature. The sporophores of *Lenzites saepiaria* are xerophytic, and will remain viable in a dried condition for some time. Buller ('09, p. 111) found them to recover after four months of desiccation, while Falck ('09) found that in one case a sporophore was still feebly viable after a year and nine months of desiccation. In my work where the tissue method was employed, the sporophores were broken open, and small pieces of the tissue from the interior were gouged out by means of a sterilized scalpel. These pieces of tissue were quickly flamed and transferred to agar slants in tubes, after dipping in sterile distilled water to moisten. The agar used was Thaxter's glucose-potato-hard agar, made up as commonly employed in this laboratory in the following way: Two hundred grams of potato were cooked for about one hour in a liter of tap water. The potato water strained off was restored to a liter. To this were added 20 grams of glucose and 30 grams of agar, and the mixture autoclaved for 20 minutes to dissolve the agar, which was then tubed, sterilized, and slanted. This is a very good medium for the growth of *Lenzites.*

The tissue transfers grew well, but practically one-half of them were contaminated, since difficulty was found in securing a piece of tissue from the interior of such thin sporophores without contamination.

(2) The spore method was more frequently used in this work. Buller ('09) discovered the remarkable fact that many xerophytic fungi which have been preserved dry for several months or even years may be revived by moistening, when spore expulsion will be resumed and will continue for several days or weeks (according to the specificity of the organism), even after the plants have been dried and revived several times in succession. In the same year, however, Falck reported his work on the desiccation of sporophores of *Lenzites saepiaria, L. abietina,* and *L. thermophila,* in which he used this rejuvenescence due to moisture as an index of viability. The spore method is used considerably in forms with thin tissues, having been employed by Ferguson ('02), Falck ('02), Lyman ('07), Münch ('09), and others.

My procedure differs from those of previous workers in some details, which are given here. To obtain the basidiospores the sporophores were rinsed twice in sterile distilled water in large-sized Petri dishes. This removes some of the bacteria and spores of foreign fungi. After this washing process the sporophores were allowed to stand in sterile distilled water for about two hours, so that they were thoroughly saturated. They were removed with sterile forceps and sponged off with Scott's tissue toweling which had been previously sterilized, and were then placed, hymenium downward, in large, dry, sterile Petri dishes. After 24–48 hours the sporophores had discharged enough spores to make a white spore print. The moisture in the sporophores serves two purposes other than reviving the tissues. It keeps the air in the dish sufficiently humid to prevent too rapid desiccation, and it also tends to hold foreign spores to the surface of the sporophores. The latter is beneficial in securing a fairly pure dispersion of spores.

The spore dispersion was made in sterile distilled water. Test-tube water-blanks were prepared and sterilized. Several loopfuls of sterile water were transferred to the spore print by means of a platinum loop. By stirring a little with the loop the spores were so dispersed that when a loopful of the spore suspension was transferred to the water-blank a cloudy streak was produced. Three or four such loopfuls of the spore suspension were transferred to a water-blank, which was well shaken by rolling between the palms of the hands. Two or three loopfuls were transferred to each of several tubes of melted agar, and poured plates were made as in the usual bacteriological method. Plates made in this way with the 3 per cent agar mentioned above were surprisingly free from bacterial contamination but contained a few scattering colonies of foreign moulds. The colonies produced by the germinating spores of *Lenzites* were so characteristic, however, and so generally scattered over the plates that they soon became easily recognizable. Individual colonies from the plates were transferred to agar slants, a quantity of cultures being obtained.

CHARACTERISTICS OF THE MYCELIUM IN CULTURE

Lenzites saepiaria was kept in cultures on three types of media, i.e., Thaxter's glucose-potato-hard agar described above, yellow pine sawdust in Erlenmeyer flasks, and pine blocks in jars and bottles. The mycelial characters on wood are somewhat different from those on agar.

Polymorphism.—Polymorphism in the *Basidiomycetes* has been reported by many early investigators, and in late years especially by Falck in 1902 and Lyman in 1907. Lyman reviews the literature dealing with the occurrence of oidia, chlamydospores, and conidia in certain *Polyporaceae,* and in his own work finds that many of the *Hymenomycetes* possess these several ways of reproducing vegetatively. In 1909 Falck published a monograph on *Lenzites* in which he describes minutely the morphology and physiology of the mycelium of *L. saepiaria.* The polymorphism of this fungus is extremely interesting and characteristic, the pure cultures of the mycelium being almost sufficient to identify the fungus.

The mycelium is white at first, but with age the aërial part becomes a reddish brown or sepia color. When the mycelium grows out from an inoculum on agar, there is a submersed mycelium which is a forerunner of the superficial. From the latter there arises a woven mat of aërial hyphae which take on the sepia color with age. The hyphae are very much septate, and clamp connections are quite common. These vary from the ordinary clamp connections through all stages to the medallion mycelium, as Falck calls it, which is found only in the wood and sawdust cultures.

Falck divides the oidia into primary, secondary, and tertiary. The primary oidia are those formed when the whole superficial mycelium breaks up into chains of spore-like cells. The secondary are those produced at the tips of branches of the superficial mycelium. These may be abnormally swollen tips or very short chains from lateral branches. The tertiary oidia are the most common and appear on the aërial hyphae. The hyphae generally break at clamp connections to produce this type. The chlamydospores or gemmae are nothing more than swollen vegetative cells of the hyphae, which act as conidia when isolated. In the superficial mycelium they are larger than where submersed in agar, and are often found in the medallion mycelium in the decayed wood.

In the cultures on blocks of pine wood the mycelium spreads well if the blocks are fairly well saturated with water. As the moisture disappears the mycelium usually penetrates, and the superficial mycelium dries and vanishes. In this later stage of growth, however, it is difficult to see whether the blocks are well infected or whether the fungus has failed to enter and thus has dried down. In the woody tissues the hyphae extend lengthwise of the tracheids as a general rule. The medullary rays are usually full of matted hyphae. When passing from one tracheid to another the hyphae penetrate the pits. Very infrequently, however, they penetrate the walls of the spring wood, and in such cases are constricted in the tiny perforation, but are swollen, forming a callus on either side of the wall. When two active hyphae come together laterally they fuse, and the whole often forms an anastomosing network. The medallions are extremely common.

CHARACTERISTICS OF THE SPOROPHORES IN CULTURE

Well-differentiated sporophores were formed on sawdust and pine blocks, and in a few cases sporophores occurred on agar. The earliest fruiting bodies appeared about seven months after the cultures were inoculated, and were thelephoroid or staghorn-like in shape. The flattened projections bear the hymenium on both sides. It is composed of clavate paraphyses with the typical four-spored basidia. Still better-differentiated sporophores were produced in from eleven to twelve months, their form depending upon the surface of the substrate. On a horizontal surface they are almost sessile and hemispherical, with the upper surface composed of tiny daedaloid pores, while the under surface has the typical lamellate hymenium of *Lenzites.* When fruit bodies appear on the side of a block they tend toward the bracket form, but have hymenium above and below, the upper poroid, the lower lamellate or hydnoid. Plate 8 shows sporophores both on sawdust and on pine block cultures.

Increase in sporophore-producing capacity.—The basidiospores from one of the sporophores produced in pure cultures were caught in a sterile Petri dish and plated out in agar as described above. There was a large per cent of germination. The pure culture tubes made from these germinated spores produced sporophores on agar in eight weeks after the transfers were made. This is the only instance where I secured sporophores of *Lenzites saepiaria* on agar. It seems plausible to conclude that by growing the fungus in pure cultures the ability to produce sporophores in cultures is increased in the following generation.

FACTORS INFLUENCING THE GROWTH AND METABOLISM OF THE FUNGUS

In this connection four different factors are discussed. These are (1) the relation to the reaction of media, (2) temperature relations, (3) the water and oxygen content of the substrate, and (4) resin on wounds and in the wood.

(1) The relation to the reaction of media, with special reference to chemical composition, i. e., source of carbon and other nutrients for best germination and growth, has been amply considered by Rumbold ('08), and by Falck ('09) in his monograph on the Lenzites rots. Thus it was not considered necessary to dwell upon these factors further than is reported elsewhere in this paper. However, in making a new supply of cultures of *Lenzites saepiaria, Fomes pinicola, Polystictus hirsutus, Polyporus lucidus* and others, I found that after transferring to the new medium the fungi would not grow. On testing with litmus this medium proved to be slightly alkaline. A readjustment of the reaction to slight acidity yielded a suitable medium for these forms. Rumbold ('08) also found that *L. saepiaria* is very sensitive to alkaline media, and Spaulding ('11, p. 19) found that "a number of experiments uniformly gave the same results with this species. It was found that even with one-fourth of one per cent of sulphuric acid it grew luxuriantly."

(2) In Falck's ('09) paper temperature relations are discussed at length. He shows that *L. saepiaria* has a growth

range from a minimum of 5°C. to a maximum of 44°C., with 35°C. as an optimum temperature. This optimum holds for the strain of this species that I used, but the total range of temperature was not determined. A question still remains whether the optimum temperature for growth is an optimum for the complex of enzyme activities which take place during decay. With this in mind the cultures were maintained at 25–30°C., since these temperatures are at least conducive to growth, and enzyme action *in vitro* is rapid within these limits.

(3) Given some specific wood as a suitable substrate and a favorable temperature, then the growth of *L. saepiaria* will be related to such other factors as porosity, water content, oxygen tension, and abundance of stored starches or other food materials in the wood. The oxygen content of wood is necessarily related inversely to water content, provided there is water in excess of that imbibed by the tracheid walls. Variation in the imbibed water must influence the degree of humidity of the air in the lumen of the tracheids, and doubtless the humidity of this enclosed air plays a rôle in the growth of the fungus through the wood. Another factor which influences the oxygen content of wood is the average size of the cell lumen. This decreases in size from the spring to the late summer wood, for in the latter the lignification increases the thickness of the walls at the expense of lumen capacity. High specific weight is directly related to the amount of summer growth (Johnson, '93). This necessarily means that as the specific weight of the wood increases the oxygen content would be decreased.

Münch ('09) has shown by numerous experiments with various forms of wood-destroying fungi that air content is an important factor influencing the entrance of fungi. The greater number of the forms which he used have a high air requirement. The quantity of wood fibre is also important. He mentions the fact that narrow annual rings are more resistant than broad ones, because there is less capacity for air in the narrower. In specimens of wood where only some rings are decayed the decayed rings prove to be the more porous ones. This shows that a certain undetermined minimum of oxygen will prevent the growth of certain fungi. Exceptions to this, however, may be numerous. For instance, Münch found that *Armillaria mellea* is not dependent on air in the substrate. The rhizomorphs of this organism are of such a structure that they conduct air into water-saturated tissues. Doubtless there are other forms which have the power to thus conduct air to parts where there is a paucity of air.

Undoubtedly, high water content will inhibit the entrance of certain fungi, but as soon as there is a paucity of water the tissues are as susceptible as ever, for there is no change in the properties of the host tissues themselves. Thus, an increased water content as a factor in the immunization of a host plant against disease (as Münch would lead one to think) is not compatible with present-day ideas of immunity.

Appel ('15) concurs in Münch's idea that the paucity of air due to high water content may be made an effective method in the control of certain plant diseases. He applies this to die-back diseases of trees due to species of *Valsa* and other fungi. He says:

> "When such diseases occur, you will find the cause in defective irrigation methods, which may be remedied by changing the irrigation system. It is of the greatest importance that the land be irrigated at the time the trees contain less water and plenty of air, and that the next irrigations be made in time to prevent an excessive decrease of the water in the tissues."

Further, he states that the same principle may be found to be applicable to bacterial diseases of trees, especially *Bacillus amylovorus,* and finally remarks:

> "It may be possible that not only trees, but also herbaceous plants, show relations between fungous growth and air content. I think it must be so for the organisms which cause the wilt diseases and the rhizoctonia disease of the potato, both of which have a high air requirement. . . . . . Though caused by a fungus, the production of conditions favorable to the progress of the disease [*Rhizoctonia*] is attributable to irrigation."

The writer believes that the close application of the work of Münch given by Appel to such diseases as those caused by

Bacillus amylovorus and *Rhizoctonia* is hardly acceptable without adequate specific data for each of the organisms concerned.

Speaking of the fungous diseases in the tropics, Westerdijk ('15) says:

> "The heavy rainfalls, combined with the abundant transpiration—owing to the intense heat, must cause a high water-content and a small air-content, of the wood-vessels of the trees, thereby making a substratum poor in air. This fact, combined with the high temperature, would explain the rare occurrence of *Hymenomycetae* and other wood-destroying fungi in the tropics."

A certain balance between water and oxygen is necessary, and this varies according to the specificity of the organism. Just what percentage of water in the cell walls and oxygen in the lumen of the wood fibres are necessary for the entrance of fungi are undetermined factors, but we know that both are necessary. Well-seasoned wood is very durable as long as it is kept dry. On the other hand, upon damp wood spores of fungi germinate and penetrate readily. In speaking of *Lenzites spp.* Falck ('09, p. 223) says that the spores germinate with every rain, then there forms a small colony from which hyphae enter the wood substance. In such colonies are found the typical medallion mycelium which endures dry periods, and which after a thorough saturation with water is again able to continue its life activities undisturbed.

Wehmer ('14) found that bits of mycelium of *Merulius lacrymans* transferred to air-dried blocks would not grow at room or cellar humidity. When the blocks were well saturated with water better results were obtained for mycelial growth, but decay was not evident in all cases. As decay by *Merulius* spreads the moisture content increases, decayed wood showing 25 per cent hygroscopic water in damp air, where sound pine holds but 15 per cent.

During the course of my work with *Lenzites* grown on pine blocks some interesting facts were noted concerning the growth and decay as they were influenced by the water and oxygen content of the wood. Blocks which were sterilized without excess of water were inoculated and enough water added from time to time to keep the humidity high, but the mycelium did not spread far from the place of inoculation. Where the blocks were kept saturated until after inoculation the mycelium grew rapidly over the surface, no matter what the nature of the block. As the water evaporates the mycelium penetrates, and the superficial growth dries down and disappears. The interior of the blocks, however, holds moisture for a longer time than the surface, and in such a proportion to the wood fibre that the nearest to optimal water content is reserved in the interior. This is shown by examples of Lenzites rot wherever it is found in nature, as well as in pure cultures. Blocks that show a reduction in weight (due to decay) of from 40–60 per cent show internal decay, with a crust of fairly sound wood over the surface. If two blocks fit rather closely together during incubation the surfaces in contact may be decayed.

A large series of blocks inoculated with *L. saepiaria* were kept saturated for a year, and they were reduced very little by the fungus. The reduction was all superficial and appeared as a "scorching" of the surface. Microscopic examination showed that the hyphae penetrated to a depth of but three or four tracheids. Plate 8, figs. 13–17, shows a series of blocks having this superficial "scorching," and figs. 8–12 show the internal decay mentioned above. From these observations it is apparent that the oxygen requirement of *L. saepiaria* is low. A certain percentage of water is a necessary factor, but total saturation is injurious to the fungus because of the paucity of oxygen. The optimum, maximum, and minimum percentages of air and water have not been determined.

From the above observations it will be seen that any factors influencing the proportion of water and air are of great importance. Seasonal cracks, due to drying, of ties and other structural timbers afford an entrance place for the fungus and the necessary air, and usually the decay is found in radial blotches when the end of an infected timber is observed.

(4) When coniferous trees are wounded in one way or another the majority of them exude pure resin from the

bark, and this seals the wounds to the exclusion of the fungus. We have found that the mycelium of *L. saepiaria* will not grow on 100 per cent resin plates because of the lack of nutrition, nor will the spores germinate on such a medium. In living trees, then, the pure resin covering the wounds serves a twofold purpose. It mechanically prevents the entrance of the fungus, and excludes the air which might otherwise gain access to the interior of the tree more rapidly than under natural conditions. Resin does not exist in this high percentage, however, in the interior of wood, but is infiltrated into the lignified walls. Hence, there is still air in the lumen and food accessible to the fungus. Laboratory experiments reported below have shown that under these conditions the presence of resin has very little influence upon the growth of the fungus, at least up to 50 per cent resin by weight, which is considerably more than is found in any coniferous wood.

A quantity of resin was extracted by means of benzol from longleaf pine wood (*Pinus palustris*). The resin was hardened at 65°C. until it was of a constant weight. This was used in making a resin agar, the basis of which was a 4 per cent Thaxter's glucose-potato-hard agar. The powdered resin was added to the agar while warm, emulsified by vigorous agitation, and then sterilized. Sterilization may be done in the Arnold sterilizer on three successive days, or if it is necessary to sterilize but once, autoclaving is satisfactory; but when autoclaved more than once the resin seems to be acid enough to hydrolyze the agar sufficiently to keep it from hardening. If the agar is removed from the autoclave while it is still quite super-heated and vigorously agitated while it is cooled by placing under the water tap from time to time, a very satisfactory emulsion of any percentage may be obtained.

The agar was made up so as to contain 5, 10, 15, 20, etc., up to 100 per cent, of resin, which was plated out and after cooling was inoculated with squares of mycelium of *L. saepiaria* cut as suggested by Humphrey and Fleming ('15, pl. 1). The inocula were 0.8 cm. on a side. After 14 days of growth at 32°C. measurements of the diameters of the growing mycelial colonies were taken. Plate 9 shows photographs of the comparative growths. The accompanying table (table I) shows that on the 5 per cent resin agar the growth was reduced somewhat, as compared with the control containing no resin; but the growth was just about as rapid on 40, 45, and 50 per cent resin as on 5, 10, and 15 per cent, the average growth of the former three being 6.4 cm., and of the latter 6.84 cm. From 50 to 60 per cent there was an abrupt change in the growth, which showed marked inhibition from 60 to 85 per cent, while above this there was practically no growth. The fact that there was some growth up to 85 per cent resin is conclusive evidence that resin is not toxic. Of course, it could not be toxic unless soluble in water or the enzymic fluids excreted by the fungus. Its greatest inhibitive power lies in the fact that it excludes water from the substrate. The results given

TABLE I

RESULTS OF GROWING LENZITES SAEPIARIA ON PLATES OF RESIN AGAR

Per cent of resin	Diameter of growth on resin agar plates after 14 days at 32°C.				
	Plate no. 1	Plate no. 2	Plate no. 3	Plate no. 4	Averages
	cm.	cm.	cm.	cm.	cm.
0 (control)	9.0	9.0	9.0	9.0	9.0*
5	7.6	7.4	6.8	7.9	7.42
10	6.1	6.2	6.7	7.4	6.6
15	6.8	6.5	7.2	5.5	6.5
20	6.2	6.1	5.9	6.2	6.1
25	6.4	6.8	6.9	6.4	6.63
30	6.2	5.7	6.2	5.9	6.0
35	6.8	7.0	6.6	6.8	6.8
40	6.5	6.2	6.6	6.4	6.4
45	7.4	7.0	6.6	6.4	6.85
50	5.9	6.2	5.7	6.0	5.95
55	3.4	3.5	3.8	3.7	3.6
60	1.3	1.5	1.8	1.4	1.5
65	1.8	1.4	1.7	1.5	1.6
70	1.8	1.6	1.7	1.3	1.6
75	1.6	1.7	1.8	1.7	1.7
80	1.4	1.5	1.1	1.2	1.3
85	1.5	1.6	1.4	1.4	1.5
90	0.8	0.8	0.9	1.1	0.9
95	0.8	0.8	0.8	1.2	0.9
100	0.8	0.8	0.8	0.8	0.8

* The diameter of the inoculum in each case was 0.8 cm., which should be subtracted from the above values in order to obtain the actual growth.

here are merely indicative of the true conditions as they exist in nature, since the nutrition in the two cases is different. We know from the work done by Le Renard ('12) that the composition of the nutrient medium used in such Petri dish plates has a marked influence on the effect of any toxic substance present; that is, if the nutritive elements present are varied in quantity there is a change in the effect of the toxic substance on the growth of the organism.

Resin determinations have been made on approximately 450 series of pine blocks, the whole comprising about 3000 samples which were placed in cultures of *L. saepiaria.* The reduction in weight of these blocks, after incubation for one year, varies considerably, but the results so far taken tend to show that the influence of resin on the decay by this fungus is exceedingly erratic. This will be reported in a second paper.

ENZYME ACTIVITY IN LENZITES SAEPIARIA

Work on the enzyme activity of the wood-destroying fungi is comparatively meagre. This is especially the status of the cytolytic investigations. There are very few papers wholly devoted to enzymes of higher fungi. In 1895 Bourquelot and Hérissey investigated the enzymes from the juice of the sporophores of *Polyporus sulphureus.* The enzymes were precipitated with alcohol. Czapek, in 1899, found in natural infections of *Merulius lacrymans* an active principle capable of liberating from lignin the substance which gives the lignin reactions in alcoholic extracts. This substance, which is discussed more fully later in this paper, he called "hadromal," and the enzyme capable of liberating it was called "hadromase."

Two years later Kohnstamm ('01) applied Buchner's "Dauerhefe" method to the sporophores and mycelium of *Merulius lacrymans* and *Armillaria mellea.* He obtained evidence of the presence in these fungi of diastatic, proteolytic, glucoside-splitting, and cellulose-hydrolyzing enzymes. Buller ('06) tested out the juice expressed from the sporophores of *Polyporus squamosus* and obtained positive evidence of the presence of eight enzymes. Reed ('13) grew *Glomerella rufomaculans* in cultures of nutrient solutions, and from the dried fungous mat was prepared a fine, enzyme-containing meal which was tested on various substrates.

The few papers mentioned are the main ones dealing entirely with enzyme activity in fungi which attack wood. Many other investigators have inferred *a priori* that many enzymes, especially cytolytic, are active agents in the metabolism of this group of fungi, and scattering references of this sort are numerous. It is of interest to observe that although we have every indication to direct us to believe that cytases are present in wood-destroying fungi, yet their presence has been demonstrated only indirectly, i.e., by histological methods. This may be due to the fact, as will be pointed out later, that a majority of the investigators who found no wood-destroying ferment used the fruiting bodies in their experiments instead of the active, vegetative mycelium. More detailed discussions of the results of these various investigators will be given below in connection with the various groups of enzymes considered in this paper.

In view of the status of our knowledge of the enzymes concerned in the destruction of wood, I undertook an investigation to compare in a qualitative way the enzymes of the mycelium and sporophores of *Lenzites saepiaria.* In certain cases only have quantitative results been obtained.

METHOD OF GROWING MYCELIUM FOR EXTRACTION

Sawdust of *Pinus palustris, P. echinata,* and *P. Taeda* was placed in flasks of 1000 and 500 cc. capacity. The sawdust was moistened with distilled water, after which the flasks were plugged and sterilized in the autoclave at 20 pounds pressure for 45 minutes. After cooling, the sawdust was inoculated with the mycelium from agar slants. As the mycelium grew into the sawdust there was a darkening of the wood similar to that noticed in the "rot" produced in nature by this fungus. In the course of time the sawdust became a dark brown color.

After about 7 months some of the flasks were emptied, and the sawdust with the mycelium was dried by means of an electric fan blowing a draft of warm air (about 30°C.) over it. After being dried the sawdust could be crushed to powder between the fingers. This dry sawdust was ground to a very fine powder in an ordinary mill. A tared amount of the powder was transferred to a clean liter flask, and about 4 parts of water by volume were added with about 1 per cent chloroform. This was allowed to stand for 16 hours for the extraction of enzymes. Then the solution was filtered off through a Buchner funnel, and the enzymes precipitated from the filtrate by the addition of 3 volumes of 95 per cent alcohol. The precipitate was collected on a filter paper in a Buchner funnel, and the paper allowed to dry at room temperature. These filter papers were kept in glass-stoppered bottles for future use.

When the enzyme preparation was to be used the filter paper was soaked in such a quantity of water that each cubic centimeter of the resulting enzyme "dispersion" was equivalent to 1.5 grams of the original sawdust powder. In the following work 2 cc. of this dispersion were used in 10 cc. of the respective substrates.

PREPARATION OF SPOROPHORES FOR EXTRACTION

Sporophores of *Lenzites saepiaria* were collected by the writer at Leeper, Missouri. Only the viable, light sepia-colored specimens were used in the enzyme work. The tissue of the sporophores, after grinding, was treated in the same way as described above for the mycelium in sawdust. The enzyme preparation was secured in the same way, and 1 cc. of the enzyme dispersion in water had the value of 1.5 grams of the original sporophoral meal. Unless otherwise specified in the following pages "mycelial meal" will refer to the original powdered sawdust including mycelium before extraction, "sporophoral meal," the original ground sporophores before extraction, "mycelial dispersion," the dispersion of enzymes extracted from the mycelial meal, and "sporophoral dispersion," the dispersion of enzymes extracted from the sporophoral meal.

ENZYMES OF LENZITES SAEPIARIA

ESTERASES

By the action of esterases the esters of the fatty acids are saponified and are thus resolved into their constituents, alcohols and fatty acids. It is thus possible to recognize and measure the activity of the enzymes in decomposing esters by determining the acidity of the substrate quantitatively by titration against alkali.

The presence of fatty globules in the hyphae of fungi has led to the investigation of the enzymes capable of accomplishing their hydrolysis. Biffen ('99) found a fat-destroying fungus belonging to the *Hypocreales* which grew luxuriantly on the endosperm and milk of the cocoanut. All cultures of the fungus showed that the fats became emulsified, and the substrate became increasingly acid with continued growth and had a pleasant ethereal odor something like that of amyl butyrate. On triturating the mycelium with kieselguhr, and filtering under pressure, he obtained an extract which decomposed both cocoanut oil and monobutyrin. Buller ('06) found that 10 cc. of the juice from the sporophores of *Polyporus squamosus* hydrolyzed 43 per cent of a 1.84 per cent ethyl acetate solution in 330 hours. He mentions "that when spores of *Polyporus squamosus* are allowed to dry for several days, many of them develop large fat drops. On germination of the spores in malt-wort extract these drops disappear. Perhaps this is due to the action of lipase." Bayliss ('08) was not able to demonstrate the presence of lipase in *Polystictus versicolor*. Dox ('10) has reviewed the literature concerning the filamentous fungi which split the fats. Reed ('13) found that the enzyme powder prepared from *Glomerella rufomaculans* split ethyl acetate and ethyl butyrate, but that the increase in acidity was much greater where ethyl acetate was used as the substrate.

The presence of oil globules in the spores and mycelium of *L. saepiaria* led to the investigation of the power of the fungus to utilize such substances. Thus, experiments were conducted using as substrates the following: olive oil emulsion, triacetin, methyl acetate, ethyl acetate, and ethyl butyrate.

Olive oil emulsion was made according to Bloor's ('14) method, which was previously reported from this laboratory by Davis ('15). Ten cubic centimeters of olive oil were dissolved in hot, absolute alcohol. This was run through a hot funnel to which was attached a piece of glass tubing drawn out to a very fine jet. The fine stream of oil in alcohol was run into 100 cc. of cold distilled water, which was constantly stirred. The milk-white emulsion was finally boiled to drive off the alcohol, then was diluted to 500 cc. with distilled water.

The other substrates were made up in 1 per cent solutions, and if kept any length of time toluol was added as an antiseptic. All substrates were used according to the following example and were always set up in duplicate:

(1) 25 cc. ethyl acetate + 5 cc. enzyme dispersion + toluol.

(2) 25 cc. ethyl acetate + 5 cc. enzyme dispersion (autoclaved) + toluol.

(3) 25 cc. ethyl acetate + 5 cc. water + toluol.

(4) 25 cc. ethyl acetate + equivalent weight meal + toluol.

(5) 25 cc. ethyl acetate + equivalent weight meal (autoclaved) + toluol.

The results show that the lipolytic action on neutral fats is very slight, if any, but that on the esters of the lower fatty acids it is more marked. As Reed ('13) observed for *Glomerella rufomaculans,* here the acetates are better substrates than the butyrates. Methyl acetate is more strongly hydrolyzed than ethyl acetate.

Table II shows the results of the action of the esterases in the mycelial meal and the enzyme dispersion from the mycelium. These cultures were incubated for 24 days at a temperature of 25–30°C. Table III shows the lipolytic action in the sporophores under the same conditions. Since the mycelial meal includes the sawdust upon which the mycelium grew, the results obtained for the mycelium and sporophores are not comparable, but from the results on methyl acetate it can be readily seen that the esterase activity in the mycelium is stronger than in the sporophores.

TABLE II

ACTION OF ESTERASES OF THE MYCELIUM UPON VARIOUS SUBSTRATES

Substrate	Form of enzyme material	Number cc. N/20 NaOH to neutralize 10 cc. substrate	
		Gross	Net
Methyl acetate	Enzyme dispersion	2.95	2.30
Methyl acetate	Enzyme dispersion (autoclaved)	0.65	....
Ethyl acetate	Enzyme dispersion	1.60	0.70
Ethyl acetate	Enzyme dispersion (autoclaved)	0.90	....
Ethyl butyrate	Enzyme dispersion	0.15	0.05
Ethyl butyrate	Enzyme dispersion (autoclaved)	0.10	....
Olive oil emulsion	Enzyme dispersion	0.35	0.10
Olive oil emulsion	Enzyme dispersion (autoclaved)	0.25	....
Olive oil emulsion	Equivalent weight of meal	7.57	0.07
Olive oil emulsion	Equivalent weight of meal (autoclaved)	7.50	....
Triacetin	Equivalent weight of meal	9.08	0.48
Triacetin	Equivalent weight of meal (autoclaved)	8.60	....

TABLE III

ACTION OF ESTERASES OF THE SPOROPHORES UPON VARIOUS SUBSTRATES

Substrate	Form of enzyme material	Number cc. N/20 NaOH to neutralize 10 cc. substrate	
		Gross	Net
Methyl acetate	Enzyme dispersion	7.10	2.30
Methyl acetate	Enzyme dispersion (autoclaved)	4.80	....
Methyl acetate	Equivalent wt. meal	12.30	5.90
Methyl acetate	Equivalent wt. meal (autoclaved)	6.40	....
Ethyl acetate	Enzyme dispersion	4.55	1.25
Ethyl acetate	Enzyme dispersion (autoclaved)	3.10	....
Ethyl acetate	Equivalent wt. meal	9.40	4.70
Ethyl acetate	Equivalent wt. meal (autoclaved)	4.70	....
Ethyl butyrate	Enzyme dispersion	0.42	0.07
Ethyl butyrate	Enzyme dispersion (autoclaved)	0.35	....
Ethyl butyrate	Equivalent wt. meal	2.65	0.95
Ethyl butyrate	Equivalent wt. meal (autoclaved)	1.70	....
Olive oil emulsion	Enzyme dispersion	1.10	0.25
Olive oil emulsion	Enzyme dispersion (autoclaved)	0.85	....

MALTASE

Of the disaccharases, I tested for maltase, lactase, and invertase. The occurrence of maltase in some of the lower fungi is of special interest and importance, but the literature has been adequately discussed by Dox ('10). In the higher fungi it has been demonstrated in the sporophores of *Polyp-*

orus sulphureus by Bourquelot and Hérissey ('95), and by the writer it has been found both in the mycelium and sporophores of *L. saepiaria.*

In our experimental work a 1 per cent solution of maltose was used as a substrate. Ten cubic centimeters of this were placed in each of 6 test-tubes. To each of 2 of these were added 2 cc. of the mycelial dispersion, to each of 2 others, 2 cc. of the mycelial dispersion which had been autoclaved to kill the enzymes, and to each of the remaining were added 2 cc. of distilled water. To all were added a few drops of toluol as an antiseptic. A comparable series of experiments was set up using sporophoral dispersion. All were incubated at 25–30°C. for 2 weeks, after which time they were tested for reducing sugars by Shaffer's method ('14). Maltase reduces Fehling's solution, but when it is hydrolyzed each molecule yields 2 of glucose which would reduce almost twice as much Fehling's solution. Table IV shows that there was considerably more reduction in the "regular" tubes than in the autoclaved and the water controls. The fact that there is more net reduction due to maltase in the sporophores than in the mycelium is not of any significance when the amount of sawdust in the mycelial powder is considered.

TABLE IV

SHOWING THE ACTION OF MALTASE IN LENZITES SAEPIARIA AFTER TWO WEEKS AT 25–30°C.

Amount of enzyme dispersion in 10 cc. of 1 per cent maltose	Reducing sugars as glucose in 5 cc. of substrate		Net due to hydrolysis by maltase	
	Mycelium	Sporophore	Mycelium	Sporophore
	mg.	mg.	mg.	mg.
2 cc.	40.40	39.24	6.28	18.61
2 cc. (autoclaved)	34.12	20.63	......	....
2 cc. dist. water	26.16	19.72	......	....

LACTASE

Lactase has never been reported from the higher fungi. In dealing with lactase the same procedure was carried out as for maltase, except that a 1 per cent solution of lactose was used for a substrate. There was no indication of the presence of lactase either in the mycelium or the sporophores.

INVERTASE

Although invertase has been repeatedly demonstrated in lower fungi, especially yeasts, *Aspergillus* and *Penicillium,* its presence has seldom been noted in the higher forms. Bayliss ('08) found it in the sporophores of *Polystictus versicolor,* and it is undoubtedly present both in the mycelium and sporophores of *L. saepiaria.*

To demonstrate the presence of invertase a 1 per cent solution of sucrose was used as a substrate. To 10-cc. portions of this were added 2 cc. of the enzyme dispersions, enzyme dispersions autoclaved, and 2 cc. of distilled water, as in the maltase experiments. After 4 hours there was distinct reduction of copper oxide from Fehling's solution in the regular tubes, but the autoclaved controls and the water controls showed none. This was perhaps more evident in the sporophoral dispersion.

Having demonstrated the presence of invertase both in the mycelium and sporophores, some quantitative studies were made. The fungus was successfully grown upon a substrate of carrot juice. The carrot juice cultures were made in Erlenmeyer flasks and inoculated with oidia from cultures grown on pine sawdust. The oidia were dispersed in sterile, distilled water-blanks from which the inoculations were made. After 2 weeks of growth the mats of mycelium were removed from the flasks and dried on filter paper at a temperature of about 35°C. This dried mycelium was ground to a fine powder in a mill and kept dry in a glass-stoppered bottle. This same powder was used for quantitative determinations of diastatic action reported later in this paper.

The experiments were conducted as follows: To 50 cc. of a 1 per cent sucrose solution 2 grams of fungous powder were added, with about 1 per cent toluol as an antiseptic. As a control on this, other experiments were prepared in the same way after the fungous powder had been autoclaved to kill the enzyme. The sucrose solution was also used alone. These were all set up in duplicate, and at the same time parallel experiments were set up with sporophoral meal in the same way. They were allowed to remain at room temperature (about 27°C.). After a period of 3 hours one set of cultures was killed in the autoclave by allowing the pressure to come to 10 pounds. The duplicate set remained in incubation for 6 hours and then was killed in the same way. After killing the substrates were filtered, and the invert sugars were determined by the Shaffer method. Table v gives these quantitative results. These results show definitely that the greater invertase activity is in the vegetative part of the fungus, the inversion taking place over twice as rapidly in the mycelium as in the sporophores. In collecting such sessile sporophores as those of *L. saepiaria* it is difficult at times to remove the fruiting body without taking with it some superficial mycelium. It may be that if only the marginal portions of the sporophores were used in this work there would be a greater difference in the activity between fruiting body and mycelium.

TABLE V

QUANTITATIVE STUDY OF THE INVERTASE ACTIVITY IN MYCELIUM AND SPOROPHORES

Amount of fungous powder in 50 cc. of 1 per cent sucrose solution	Reducing sugars as glucose in 10 cc. of substrate				Net invert sugar due to invertase activity			
	After 3 hrs. incubation		After 6 hrs. incubation		After 3 hrs. incubation		After 6 hrs. incubation	
	Mycelium	Sporophore	Mycelium	Sporophore	Mycelium	Sporophore	Mycelium	Sporophore
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
2 grams	7.52	2.88	17.19	5.16	5.44	2.25	15.05	4.53
2 grams (autoclaved)	2.08	0.63	2.14	0.63	......	......	......	......
Sucrose solution alone	Negligible	......	Negligible	......	......	......	......	......

RAFFINASE

A carbohydrase which transforms the trisaccharide raffinose into fructose and melibiose was demonstrated. To 10 cc. of a 1 per cent solution of raffinose were added 2 cc. of enzyme dispersion and toluol as an antiseptic. Controls were prepared as in previous experiments. After 48 hours Fehling's solution was strongly reduced in all but the controls. This was true both for the enzyme preparations from the mycelium and the sporophores. This gives evidence of the presence of raffinase in *L. saepiaria.*

Dox ('10) has reviewed the literature as to the occurrence of this ferment in *Aspergillus* and *Penicillium.* He found that mould powder of *Penicillium Camemberti* hydrolyzed raffinose, and that varying the source of carbon in the substrate exerted an influence on the amount of raffinase produced. A significant fact brought out is that lactose and sucrose yielded a larger quantity of raffinase than did other carbohydrates, and these two disaccharides, it is to be noted, contain two of the hexoses found in raffinose; that is, lactose on hydrolysis yields galactose and dextrose, and sucrose yields dextrose and levulose.

The presence of raffinase in higher fungi has not been demonstrated before, as far as the author is aware.

EMULSIN

The presence in plants of an enzyme capable of decomposing glucosides has been known since 1837, and emulsin was discovered in fungi in 1893 by Bourquelot, who found it in *Aspergillus niger,* and by Gerard, who found it in *Penicillium glaucum.* Bourquelot ('94) was able to detect emulsin in many of the higher fungi found on wood. Among those tested, 34 species (mostly *Basidiomycetes*) showed the presence of emulsin, and 9 did not. None of the 9 were found on wood. It is probable that in the destruction of wood, whether frondose or coniferous, glucosides are set free. Among these are salicin, populin, arbutin, and amygdalin from frondose woods, and principally coniferin from the conifers. Before these are available as nutrients for the attacking fungus they must be acted on by emulsin, which splits the glucoside, yielding glucose which is directly assimilable.

In 1895 Bourquelot and Hérissey found that the juice extracted from the sporophores of *Polyporus sulphureus* actively digested the glucosides, arbutin, amygdalin, aesculin, coniferin, and salicin. Working on *Armillaria mellea, Merulius lacrymans,* and *Polyporus squamosus,* Kohnstamm ('01) showed that emulsin is present in the sporophores as well as in an extract of the wood decayed by these organisms. Buller ('06) found the expressed juice of the sporophores of *Polyporus squamosus* to act similarly toward amygdalin, while Bayliss ('08) reported that negative results were obtained using an extract of *Polystictus versicolor.* Reed ('13) reported it from the mycelium of *Glomerella rufomaculans.*

In my experiments a 1 per cent solution of amygdalin was used as a substrate. Ten cubic-centimeter portions of this were placed in test-tubes, and 2 cc. of the enzyme dispersion were added to 3 of these and 1 was boiled. In another, 10 cc. of the amygdalin solution were diluted with 2 cc. of distilled water as a control. To all was added toluol as an antiseptic. All were incubated at 25–30°C. for 3 days. After incubation the two regulars reduced Fehling's solution, gave a strong odor of benzaldehyde and the Prussian blue test for hydrocyanic acid. The boiled control and water control gave none of these tests. The Prussian blue test was not quite as pronounced in the sporophoral as in the mycelial material.

The biological importance of the presence of emulsin in *L. saepiaria* is interesting, since we know that the pine, upon which the fungus grows most readily, contains coniferin. When coniferin is hydrolyzed by emulsin it yields glucose and coniferyl alcohol. The latter, through the action of oxidases, yields vanillin. Glucose is thus made available by the action of emulsin.

Blocks of wood which had been in cultures for one year, during which period they were always saturated with water, were decayed only over the surface. When these were dried in an oven at 65°C. crystals of vanillin were collected over the surface of the blocks and the interior of, as well as about, the apertures of the oven. When the blocks were decayed under moderate moisture conditions no such sublimation of vanillin was observed. Water then may be a factor to increase this oxidation process in the production of vanillin from woody tissues; at least very moist conditions seem to aid this action or the action of emulsin in the liberation of the coniferyl alcohol.

TANNASE

Knudson ('13, '13[a]), in his two papers on the tannic acid fermentation, gives a review of the literature on this subject up to that time. All of this literature deals with the tannase found in *Aspergillus* and *Penicillium,* but no work has been done to determine tannase in the higher fungi. Knudson, however, did determine the toxicity of tannic acid for fungi, and included in his list such higher forms as *Polyporus sulphureus, P. resinosus,* and *Fomes applanatus.* On 0.25 per cent tannic acid in bean decoction he found *Polyporus sulphureus* and *P. resinosus* to grow well, but no growth was made by any of these forms on 2 per cent tannin.

TABLE VI

STUDY OF THE HYDROLYSIS OF TANNIN BY THE ENZYME DISPERSION FROM LENZITES SAEPIARIA

Amount of enzyme dispersion in 25 cc. of 1 per cent tannin solution	Gallic acid after 4 weeks' hydrolysis		Net over control	
	Mycelium	Sporophore	Mycelium	Sporophore
	mg.	mg.	mg.	mg.
5 cc. dispersion	7.39	5.88	4.1	....
5 cc. dispersion (autoclaved)	3.28	5.77	......	....
5 cc. distilled water	1.31	5.89	......	....

To determine the hydrolysis of tannic acid to gallic acid, Jean's ('00) iodine titration method was used. The tannin is precipitated with albumin and salted out with excess of sodium chloride, and the gallic acid remaining is titrated against a standard iodine solution. The value of the albumin solution in terms of the iodine must be subtracted from the gross value of the titration. The experiments follow in table VI. The increase of gallic acid here is very strong and must be regarded as evidence of tannase in the mycelium of the fungus.

DIASTASE

Hartig ('78) was the first to mention the digestion of starch by a wood-destroying fungus, *Fomes annosus,* but not until the work of Bourquelot ('93–'96) did we know that diastase is widely distributed in these higher fungi. Bourquelot's work was especially with the sporophores of *Polyporus sulphureus.* Kohnstamm ('00) demonstrated the presence of diastase in *Merulius lacrymans, Polyporus squamosus,* and *Armillaria mellea,* and in 1906 Buller verified this work with the juice from the sporophores of *Polyporus squamosus.*

Diastase was found present in both the mycelium and sporophores of *L. saepiaria.*

Potato starch was made up into a ¼ per cent paste in the manner which is commonly used in this laboratory in the advanced plant physiology course conducted by Professor Duggar. Two and one-half grams of potato starch in 150 cc. of distilled water were brought to boiling, while constantly stirred. This was transferred to a flask containing about 600 cc. of hot distilled water. The whole was boiled in a reflux condenser for about 2 hours. After cooling the paste was made up to a liter by adding distilled water. To the soluble starch thus prepared about 1 per cent toluol was added as an antiseptic.

A series of experiments was set up as for maltase but using the starch paste as a substrate. After 4 hours the mycelial dispersion showed decided indication of the presence of reducing sugars, while the sporophoral dispersion after 8 hours of incubation showed comparatively less. This work was not quantitative, however, and later experiments were conducted so that quantitative results could be taken. For this work I used the fungous powder prepared from mycelium grown on carrot juice, described under invertase. The fungous powder was used directly without extracting the enzymes. A 1 per cent starch paste was made as above described and this used for the substrate. In each experiment 50 cc. of the paste were used. To this were added 2 grams of the fungous powder and enough toluol to act as an antiseptic. Control experiments were set up in which the fungous powder had been autoclaved.

Parallel experiments were prepared using the ground sporophores. The results of these experiments are incorporated in table VII.

As was the case with the invertase, the diastatic activity shows up much more strongly in the mycelium than in the sporophores.

TABLE VII

QUANTITATIVE STUDY OF THE DIASTATIC ACTIVITY IN MYCELIUM AND SPOROPHORES

Amount of fungous powder in 50 cc. of 1 per cent starch paste	Reducing sugars as glucose in 10 cc. of substrate				Net due to hydrolysis by diastase			
	After 3 hrs. incubation		After 6 hrs. incubation		After 3 hrs. incubation		After 6 hrs. incubation	
	Mycelium	Sporophore	Mycelium	Sporophore	Mycelium	Sporophore	Mycelium	Sporophore
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
2 grams powder........	10.67	3.18	24.68	7.96	8.50	2.39	22.54	7.20
2 grams powder (autoclaved).............	2.17	.79	2.14	.76	......	......	......	......
Starch paste alone......	Negligible	......	Negligible	......	......	......	......	......

CYTO-HYDROLYZING ENZYMES

Under the general term cyto-hydrolyzing enzymes, I shall consider all enzymes which attack such higher carbohydrates as lignin, cellulose, hemicellulose, and pectic bodies. In the succeeding pages the following classification of this group of enzymes will be used:

1. Ligninase, called "hadromase" by Czapek ('99), to designate the enzyme capable of splitting lignin.
2. Cellulase, the true cellulose-hydrolyzing ferment.
3. Hemicellulase, the ferment hydrolyzing the hemicelluloses.
4. Pectase, the enzyme capable of clotting the pectins.
5. Pectinase, an enzyme which hydrolyzes into reducing sugars the pectinous substances, especially the middle lamellae of plant tissues.

Ligninase.—It has often been sought to determine comprehensively the chemical composition of the non-cellulose component of woody membranes. Tiemann and Haarmann, in 1874 (cited from Grafe, '04), believed this component to be coniferin, while Singer ('82) considered lignin as a mixture of coniferin, vanillin, and wood gum, which gave a test for the aromatic aldehydes. The prompt action of Schiff's aldehyde reagent with rose aniline and sulphurous acid speaks for the occurrence of aldehyde-like substances in lignin. In fact, in 1898, Czapek succeeded in splitting off a substance from lignin by cooking it in stannous chloride solution. This substance gave the typical wood reaction when treated with phloroglucin and hydrochloric acid, and was described by him as an aromatic aldehyde which he called hadromal. According to Grafe ('04), Czapek's hadromal does not act like a homogeneous body but like a mixture of vanillin, methyl furfurol, catechol, and coniferin, which substances exist in the form of an ether-like compound with the cellulose of the cell wall, or are taken up by resin, or may be found free in slight amounts in the wood fibre. According to Czapek ('13), however, catechol and vanillin may be regarded as decomposition products of hadromal.

Other authors go only so far as to state that the substances making up lignin are intimately related to colloidal substances, and can exist neither as a chemical compound with cellulose nor as its transformed product. On the other hand, Cross and Bevan ('01) hold that lignocellulose (lignin) is a complex of normal cellulose with two bodies, the one a furfurol-yielding group, the other an aromatic or benzenoid group. Thus the chemistry of the lignocelluloses is such an open question that the decomposition products produced by enzymes from fungi are still worthy of attention.

Our knowledge of the decay of wood induced by fungi practically began with the fundamental researches of Hartig ('78) who has furnished extensive data concerning the parasitic and saprophytic fungi destroying the most important species of wood. He has shown that a radical change in the lignified walls is wrought by the fungus, and that in the first stages of decay the wood gives a blue color with zinc chloriodid, after which there is a maceration or loosening of the affected walls. That the penetration of the walls by the hyphae is the result of the excretion of active fluids by the fungus was also brought out in this work.

Not only do basidiomycetous fungi attack the lignified walls, but certain filamentous fungi cultivated on wood will penetrate. Miyoshi ('95) found that *Penicillium* and *Botrytis* penetrated the tracheids of the coniferous wood by boring through the bordered pits, while Marshall Ward ('98) showed that by growing *Penicillium* in pure culture on blocks of spruce, the fungus could bore deep into the wood by following the medullary rays in which there was reserve starch. After these more easily assimilable foods are used up the membranes themselves are attacked. Czapek ('99[a]) made similar observations simultaneously with Ward. Hartig observed that the starches disappeared very soon in the presence of the mycelium, as compared with the dissolution of the lignin, which becomes the predominant activity of the fungus.

Czapek ('99[a]) observed that, with alcohol or benzol, a great mass of hadromal can be extracted directly from wood which is destroyed by the inroads of the mycelium of *Merulius lacrymans,* as well as from the wood penetrated by the mycelia of *Polyporus adustus, Pleurotus pulmonarius, P. ornatus,* and *Armillaria mellea.* From sound wood he obtained relatively little hadromal. The alcoholic extract from the decayed wood gives an exceedingly intense red color with phloroglucin acidified with hydrochloric acid. This hadromal test is a permanent thing in all stages of the decay. The test for cellulose by the zinc chloriodid begins to appear before the dissolution of the membrane. Czapek concludes from this that through the action of the fungus the cellulose-hadromal ether is broken, and the cellulose and the hadromal are free to give their individual reactions. To demonstrate that this activity is enzymic, Czapek prepared an extract of the mycelium of *Merulius lacrymans* and *Pleurotus pulmonarius* from natural cultures. Shavings in this extract were incubated at 28°C. There was a gradual action, and after 14 days an alcoholic extract of these shavings gave a strong hadromal test with phloroglucin and hydrochloric acid. The extracted wood gave the purple reaction with chloriodid of zinc. The fungous extract lost its activity when boiled. It could be precipitated with alcohol and thus yielded a white powder. He called the active principle "hadromase." Von Schrenk ('00, p. 12) isolated the same enzyme from the mycelium of *Polyporus subacidus* growing in spruce wood.

After taking into consideration the different types of wood decay, it would seem that "hadromase" is a misnomer, since it does not act upon hadromal but upon lignin. In some forms of decay, such as the action of *Trametes Pini* upon pine (see Hartig, '78, p. 36, and von Schrenk, '00^a), the white rot of the red cedar produced by *Polyporus juniperinus* (see von Schrenk, '00, p. 9), and the action of *Thelephora perdix* on the oak, as reported by Helbig ('11) the hadromal and other bodies are split up and used by the causal organism, leaving pure white cellulose. Should an enzyme which acts on hadromal, or the soluble substances giving this red phloroglucin test ever be isolated in these cases, it would lead to a confusion in nomenclature. It is proposed that "ligninase" be used to designate the enzyme capable of splitting lignin.

A lengthy list of papers may be cited which deal with timber-destroying fungi and which refer in a direct or indirect way to the lignin-splitting enzyme. Among these publications which have not been cited above may be mentioned the work of Biffen ('01) on the biology of *Bulgaria polymorpha,* of Marshall Ward ('97) on cultures of *Stereum hirsutum,* of Buller ('05) on the destruction of paving blocks by *Lentinus lepideus* Fr., and ('06) *Polyporus squamosus* as a wood destroyer, of Falck ('09, '12) on the dry rots of *Lenzites* and *Merulius,* of Wehmer ('12, '14), and various papers by von Schrenk ('00, '00^a, '00^b, '01, '03, '14, '14^a). Whether the authors mentioned here have isolated the enzyme or not, it is probable *a priori* that the lignin-splitting enzyme is present in the fungi with which these papers deal.

Since *Lenzites saepiaria* produces a typical brown rot similar to that produced by other dry rot fungi, such as *Merulius* *lacrymans,* I was interested in the stages of decay and the enzymes involved in the destruction of the wood. In addition to the study of the enzymes, observations were made on the microchemical reactions of the sound, and various stages of the decayed, wood. These last are reported in this paper immediately after the discussion of the cytolytic enzymes, for the decay is more directly a result of these enzymes.

The following are the experiments carried out in the laboratory to determine the production of ligninase by *Lenzites,* and incidentally showing the action of cellulase.

One gram of fine shavings of the sap-wood of *Pinus echinata* was placed in each of 3 test-tubes. The shavings had previously been soaked in distilled water for 48 hours to remove as much of the soluble substances as possible, and subsequently dried. To 1 tube were added 15 cc. of the enzyme dispersion from the mycelial meal and 15 cc. of distilled water, to the second tube 30 cc. of distilled water, and to the third 15 cc. of distilled water and 15 cc. of the enzyme dispersion which had been autoclaved up to 10 pounds pressure. To all a few drops of toluol were added.

Fifteen days later the liquid was decanted from the shavings and filtered. The shavings were boiled in absolute alcohol for 10 minutes, after which the alcohol was decanted off and tested for Czapek's hadromal. With the addition of phloroglucin and hydrochloric acid, the first gave a pink color, while the second and third gave clear, colorless tests.

Some of the sections (shavings) were subsequently stained with phloroglucin and hydrochloric acid and others with chloriodid of zinc. Shavings from the first tube were stained a deep red with phloroglucin and hydrochloric acid, but with chloriodid of zinc there was a yellowish color given to all the layers of the walls. Shavings from the second and third tubes gave the same color reaction with the phloroglucin as those from the first tube, but with the zinc chloriodid the lamella next to the lumen was stained a light purple, while the outer lamellae of the wall took a yellowish color. These reactions show conclusively that hadromal is split off in the presence of an enzyme preparation from the mycelium of *L. saepiaria;*

that the same enzyme preparation is capable of hydrolyzing the free cellulose of the inner lamellae and leaving only such substances as will give a yellow reaction with chloriodid of zinc; and that the active substance in the preparation is thermolabile.

The aqueous solution, just as it was filtered from the shavings, reduced Fehling's solution; that from the second and third tubes reduced Fehling's somewhat, but in comparison not so strongly as that from the first. Thus, quantitative determinations were made to decide whether the reducing substances were due to enzyme action or possibly to the presence of other reducing substances already in the wood, as, for instance, tannins.

To this end 10 cc. of the aqueous solution from each were placed in Erlenmeyer flasks of 125 cc. capacity. A fourth determination was made as a control on the Fehling's solution. The determinations of reducing sugars were made as glucose by the Shaffer method ('14). Table VIII gives the results obtained:

TABLE VIII

REDUCING SUGARS DUE TO ENZYME ACTION OF LENZITES SAEPIARIA ON PINE SHAVINGS IN 15 DAYS

Tube number	Experiment	Reducing substances as glucose	Net above controls
		mg.	mg.
1	1 gm. pine + 15 cc. enzyme dispersion + 15 cc. distilled water	10.606	8.973
2	1 gm. pine + 30 cc. distilled water	1.492	....
3	1 gm. pine + 15 cc. enzyme dispersion (autoclaved) +15 cc. distilled water	1.633	....
4	Fehling's solution alone	Negligible	....

The results obtained here show that these reducing substances are due to enzyme action, and that they must be sugars, probably glucose and other monosaccharides. The sources of this glucose may be various. There is probably some tannin, although slight, in sap-wood. Besides this there is the hydrolysis of cellulose to reducing sugars, as demonstrated by the experiments reported later on cellulase. Then there are probably some starches of the medullary rays hydrolyzed by diastase, and coniferin hydrolyzed by emulsin, yielding glucose and coniferyl alcohol.

To amplify the results obtained above, a few pieces of a pine block which had been in pure culture of *L. saepiaria* for a period of 6 months were extracted with absolute alcohol for 10 minutes, and the amber-colored extract yielded a deep red, with the addition of phloroglucin and hydrochloric acid.

A quantity of wood from a railroad tie decayed by *L. saepiaria* was extracted with alcohol for 10 hours in a reflux condenser. The filtered alcoholic extract obtained in this way was of a deep amber color. When a small portion was diluted to 2 volumes with alcohol and tested with phloroglucin and hydrochloric acid, it gave a deep sherry-red. When the alcoholic extract was evaporated to dryness a hard amber-like residue remained. This breaks with a conchoidal fracture, and seems to be identical with Czapek's hadromal.

These results on a typical brown rot are the same as those found by Czapek ('99[a]) for the brown rot produced by *Merulius lacrymans;* that is, there is a substance, which gives the lignin reaction, set free by enzyme action. In the case of Lenzites decay, however, the cellulose disappears as rapidly as it is set free, and in this respect the action is more rapid than in *Merulius.*

The same type of experiment was repeated, using the enzyme dispersion prepared from the sporophoral meal, but there were no results worthy of mention, other than the fact that the shavings in all cultures gave the same tests as were obtained in the water control in the above series. Quantitative determinations of reducing sugars were not considered worth while, as the reduction of Fehling's solution was so slight that no visible copper oxide was thrown down.

Cellulase.—True or normal cellulose forms the groundwork of the plant cell wall in most instances. It is a complex carbohydrate of the formula $(C_6H_{10}O_5)_n$. It is distinguished by its great resistance to hydrolysis and its insolubility in most chemical solutions. To the researches of Cross and Bevan ('01[a], '06, '12), we are indebted for a great deal of our knowledge of the celluloses. A full review of the chemistry of cellulose is given by Schwalbe ('11) in a very comprehensive way. It suffices to say here that the decomposition products of cellulose are mono- and disaccharides, and the decomposition may be brought about more or less readily, according to the complexity of the cellulose molecules, by the action of acids or alkalis and by the hydrolysis due to enzyme action.

An adequate review of the literature concerning the rôle of microörganisms and filamentous fungi in the fermentation of cellulose, especially in the soil, may be had by recourse to the papers by Kellerman and McBeth ('12) and McBeth and Scales ('13). Much of the literature on early experiments with parasitic fungi and their ability to pierce the cell membrane is reviewed in the above-mentioned papers, as well as by Cooley ('14). Space will permit only a brief review of some of the more important later papers dealing more closely with the destruction of the true cellulose of wood fibre after it is set free from the lignocellulose.

Czapek ('99^a) found in the decay produced by *Merulius lacrymans* that the cellulose disappeared from the cell walls, and he concluded *a priori* that a cellulose-hydrolyzing enzyme was excreted by the fungus, although he was unable to demonstrate it experimentally. Ward ('97) observed that in the progress of wood destruction due to *Stereum hirsutum* the action proceeds from the lumen outward. The sound wood (*Aesculus*) gives no cellulose test, but the first signs of dissolution are the swelling of the layers next to the lumen and the separation of the lumen from the layers next to the middle lamellae. These swollen layers give the test for cellulose, and as they disappear the next layer becomes delignified, gives the cellulose test, and finally disappears. The middle lamellae remain untouched. Ward did not attempt to isolate the enzyme which hydrolyzed the cellulose. According to Buller ('05), wood is rotted by *Lentinus lepideus* in much the same manner as by *Merulius* and *Stereum.* It shrinks and cracks on drying, and is then very brittle and friable. The free cellulose is removed by the fungus.

It seems from experiments carried out by Kohnstamm ('01) that the juice of *Merulius lacrymans,* expressed according to Buchner's process, gave evidence of the existence of a true cellulase. He found, that after 50 hours of action of the expressed juice on leaves of *Elodea,* there was a corrosion in the form of fine lines extending out from definite spots in the walls. These streaks soon became thin, and the walls appeared to be obliquely marked with alternate light and darker lines. He mentions that this corrosion in these definite lines, which are always in the same direction, is influenced apparently by the micellar structure of the cell walls.

Buller ('06) attempted to prove the presence of cellulase in the sporophores of *Polyporus squamosus* in the following way: Thin sections of barley grains, which had been cleared of starch by means of the action of saliva, were placed in the fungous extract. No indication of cellulase was obtained, but owing to the disappearance of the cellulose from diseased wood, he assumed that the vegetative part of the fungus produced abundant cellulase.

In Falck's ('09, p. 156) discussion of the destruction of fir, pine, and spruce wood by means of *Lenzites,* he says that in the beginning of the destructive stages the lignin reactions are decreased, and in the last stages they have almost completely disappeared, but that the reactions for cellulose are negative in all stages of decay. On the other hand, in dealing with the same fungus Spaulding ('11) says that "phloroglucin and hydrochloric acid give a bright red in the rotted tissues. . . . . . Chloriodid of zinc gives a blue color only in part of the tissues in early stages of the disease, but in later ones it gives blue throughout." This would seem to indicate that the fungus had disorganized the lignocellulose, but had left the free cellulose and most of the hadromal.

Reed ('13) grew *Glomerella rufomaculans* upon a nutrient solution containing strips of filter paper. There was considerably more growth in the flasks containing cellulose than in the controls. At the end of 2 months there was somewhat more than 3 times as much dry fungous matter in the regular as in the controls. The solution gave no tests for reducing sugars, which were probably utilized by the fungus as fast as they were split off.

Wolf ('16) made poured plates of cellulose agar which he inoculated with species of *Pseudomonas, Phoma, Gloeosporium,* and *Fusarium.* He says: "There was no evidence of the production of cellulase except by *Phoma.*" Samples of normal tissues and of tissues diseased by *Phoma socia* Wolf were tested for cellulose by employing Schweitzer's reagent, and Wolf observes that "there is a slight but significant decrease in the amount of cellulose found in diseased tissues." A considerable number of determinations consistently showed that "the lesser amount of cellulose was invariably found in the diseased tissue."

Further experiments were instituted, since it was clear from microchemical tests and from the above experiments on ligninase that cellulose of attacked wood disappears. In order to test out the action of the enzyme preparations on normal cellulose, pure cellulose from two sources was prepared. One was prepared from filter paper in the way described by McBeth and Scales ('13) and later by Cooley ('14). Fifteen grams of filter paper were dissolved in Schweitzer's reagent and precipitated with dilute hydrochloric acid. After washing thoroughly with dilute acid to get rid of all of the copper and then with distilled water to get rid of all of the chlorine, a very flocculent cellulose precipitate was obtained. The water was filtered off with a Buchner funnel until the cellulose suspension was concentrated to about 500 cc. This was transferred to a liter flask which was plugged and sterilized.

Another type of pure cellulose was made from pine wood. A quantity of fine pine shavings were treated with a cold solution composed of 30 grams of potassium chlorate dissolved in 520 cc. of nitric acid (sp. gr. 1.1). The container was kept cold for 4 weeks, after which time the cellulose was washed and then precipitated from Schweitzer's reagent, as in the above case. It was sterilized and kept for future use, as was the filter paper cellulose.

Several types of cellulose agar were prepared, using both the filter paper cellulose and the pine wood cellulose. Some standard nutrient solutions for the artificial culture of fungi were used as a basis for these, such as:

(1) Richards' ('97) solution, substituting 100 cc. of the 2 cellulose suspensions for cane sugar and adding 2 grams of agar.

NH_4NO_3	1.0	gram
KH_2PO_4	0.5	gram
$Mg\,SO_4$	0.25	gram
Fe_2Cl_3	0.002	gram
Cellulose suspension	100	cc.
Agar	2	grams

(2) Cooley's solution "A," just as given by Cooley ('14, p. 306).

(3) Reed's solution, as given by Reed ('13, p. 69), with the exception of using one-half as much distilled water together with 500 cc. of cellulose suspension and 2 per cent agar.

On any of these agars *L. saepiaria* grows very slowly and without producing much of a mat. Wherever cellulose hydrolysis could be seen it was very, very slight, and this was only in cases where the pine wood cellulose was used.

Since these experiments gave such meagre results 2 per cent agars were made, using dilute extracts of carrot, turnip, and potato as bases and using cellulose suspensions in approximately the same concentrations as in the above. In this series of experiments the cloudiness of the agar due to cellulose suspension was cleared up noticeably in one case only. This was where carrot-pine-wood-cellulose agar was used. The agar was tubed and sterilized in test-tubes of 13 mm. diameter. The agar was not slanted, and after inoculation the tubes were kept in a damp chamber so that the water content of the agar would remain the same throughout incubation. These tubes were kept at a temperature of 32°C. for 4 weeks. After this length of incubation the agar had cleared to an average depth of 9 mm. in the inoculated tubes where carrot-pine-wood-cellulose agar was used. The uninoculated tubes were still uniformly cloudy. There was no hydrolysis in the tubes where carrot juice was not used. The most significant fact brought out in this series of experiments is that the pine wood cellulose is hydrolyzed by the cellulase excreted by *L. saepiaria,* while the filter paper cellulose remained untouched.

Experiments with the enzyme dispersion were set up according to the scheme as outlined in table IX. All of the experiments were set up in duplicate with both sporophoral and mycelial dispersions. Toluol was used as an antiseptic, and qualitative results were taken after 4 weeks.

TABLE IX

QUALITATIVE EXPERIMENTS SHOWING THE ACTION OF MYCELIAL AND SPOROPHORAL ENZYME DISPERSIONS ON FILTER PAPER CELLULOSE AND PINE WOOD CELLULOSE

Experiment	Reduction of Fehling's solution	
	Mycelium	Sporophore
10 cc. paper cellulose + 2 cc. dispersion..........	+ +	+
10 cc. pine cellulose + 2 cc. dispersion............	+ + + +	+ +
10 cc. pine cellulose + 2 cc. dispersion (autoclaved)	+	—
10 cc. paper cellulose + 2 cc. dispersion (autoclaved)	+	—
10 cc. paper cellulose + 2 cc. distilled water........	—	—
10 cc. pine cellulose + 2 cc. distilled water........	—	—

Since such definite results were obtained with the mycelial enzyme dispersion in the foregoing experiments, it was determined to make quantitative comparisons of the activity of cellulase in the sporophoral and mycelial tissues. To this end a carrot extract was made as described above under "invertase," and to this extract was added pine wood cellulose. This nutrient solution was placed in Erlenmeyer flasks, which were plugged, sterilized, and inoculated as described for invertase and diastase. The mats of mycelium formed after 2 weeks were removed from the flasks, dried, and ground to a fine powder which was used in the following way: To 50 cc. of the pine-cellulose suspension were added 2 grams of the mycelial powder with toluol as an antiseptic. Some of the mycelial powder was autoclaved, and controls on the above were prepared with this autoclaved powder as well as with distilled water alone. These experiments were set up in duplicate. A parallel series was prepared using the sporophoral tissue powder. After incubating these enzyme cultures at about

28°C. for 4 weeks the sugar content of the substrates was determined as glucose by the Shaffer method already mentioned. The results of these determinations are reported in table x.

In the light of these results, there is no doubt that cellulase is present in the mycelium of *Lenzites* and that its activity can be measured quantitatively. The little activity shown in the fruiting bodies is probably due to the small amount of superficial mycelium which was removed from the substrate when the sporophores were collected. It is almost impossible to get purely fruiting tissue without some such closely connected vegetative tissue, and it is also impossible to say whether or not the enzymes diffuse from the adjacent mycelium to the base of the fruiting bodies.

TABLE X

QUANTITATIVE DETERMINATIONS OF SUGAR PRODUCED BY THE CELLULASE OF LENZITES SAEPIARIA

Amount of tissue powder in 50 cc. of pine wood-cellulose suspension	Reducing sugar as glucose in 50 cc. of substrate after 28 days		Net glucose above controls	
	Mycelium	Sporophore	Mycelium	Sporophore
	mg.	mg.	mg.	mg.
2 grams powder.................	6.479	0.99	3.271	0.28
2 grams powder (autoclaved)....	3.028	0.71	....	....
Cellulose suspension alone.......	Negligible	....	....	....

A series of quantitative cellulose determinations has been made to establish further the cellulase activity in *L. saepiaria.* It was thought that possibly there might be established some relation between the percentage in reduction in weight due to decay by the fungus and in the loss in cellulose due to the cellulase, or some relation between either of these and the specific weight of the substrate. But three determinations with their controls have been made. Blocks of yellow pine, approximately 1×1×2 inches, were dried to constant weight and weighed, and the volumes were taken by immersion in mercury. From these figures the specific weight was computed. These blocks were sterilized in jars plugged with cotton, inoculated with *L. saepiaria,* and incubated under favorable moisture and temperature conditions for one year. After this period they were dried, and the percentage of reduction in weight due to fungous decay was determined. Control blocks of the same specific weight and from the same samples were kept in a sound condition.

To make the cellulose determinations the blocks from the cultures and the controls were planed into fine shavings. All of the shavings from each sample were placed in a 250-cc. Erlenmeyer flask containing about 125 cc. of the solution of potassium chlorate in nitric acid mentioned above. These were placed in an ice chest for 3 weeks, after which time the contents of each flask was diluted to about 3 liters. The diluted liquid was thus weak enough not to attack a filter paper while filtering. The liquid was filtered off through a Buchner funnel containing a tared filter paper. The cellulose thus obtained was repeatedly washed with hot distilled water until it was of a pure white color and gave a deep blue reaction with zinc chloriodid. In all cases the yield of cellulose was so clear of foreign material that it was deemed unnecessary to precipitate from Schweitzer's reagent. After drying and weighing, the percentage loss in cellulose due to the action of the fungus was determined. The different factors in this experiment are tabulated in table XI.

TABLE XI

QUANTITATIVE DETERMINATIONS OF CELLULOSE FROM BLOCKS OF PINE WOOD, BOTH SOUND, AND DECAYED BY LENZITES SAEPIARIA

Sample number	Condition	Specific weight	Original weight of wood	Weight of wood after decay	Per cent reduction in decay	Weight of cellulose	Per cent of cellulose	Per cent loss in cellulose
			gm.	gm.		gm.		
O 5	Decayed	.424	13.373	6.973	47.86	0.9563	13.714	43.057
O 5	Sound	.424	8.55	....	....	2.0592	24.084	....
F 5	Decayed	.419	12.580	8.494	32.48	1.2952	15.248	38.187
F 5	Sound	.419	9.31	....	....	2.3066	24.668	....
A 24	Decayed	.547	12.418	5.84	52.97	0.1943	3.327	75.654
A 24	Sound	.547	7.100	....	....	0.9703	13.666	....

In these 3 determinations there seems to be no definite relation between the percentage loss of cellulose and the percentage reduction in weight due to fungous decay. This may be due to different proportions of lignification in the different samples, or more likely, since other substances like coniferin, hadromal, and possibly vanillin are utilized by the fungus, the total reduction would not necessarily bear any definite relation to the reduction of any one of the complex. Helbig ('11) has made similar cellulose determinations on wood which had been altered by *Thelephora perdix.* This fungus produces a white rot, and Helbig found that with the advance of decay the percentage of cellulose increases perceptibly.

Hemicellulase.—The hemicelluloses differ from the true celluloses in that they are more easily hydrolyzed, are readily dissolved in hot dilute acids, and sometimes give a blue color with iodine. Their chemical compositions are determined by the products of their hydrolysis. They may yield dextrose, mannose, galactose, or mixtures of these, and at times xylose or arabinose. According to these decomposition products, they are differentiated into dextrans, mannans, galactans, mannogalactans, etc. Very frequently hemicelluloses are deposited upon, or as a part of, cell walls, and here play the rôle of reserve foods, especially in seeds.

Newcombe ('99) determined that the cellulose-hydrolyzing enzyme is distinct from other carbohydrases. Schellenberg ('08), through experiments on numerous fungi, also proved that pure cultures grown on substrates containing hemicelluloses and true celluloses would hydrolyze hemicelluloses but not the true celluloses. He also shows that the moulds act selectively toward the hemicelluloses from various sources. He thus differentiates between the different hemicellulases which act on various hemicelluloses, and the cellulase which hydrolyzes the true cellulose.

To ascertain whether the enzyme dispersions from mycelium and sporophores are active as hemicellulose-hydrolyzers, the endosperm of the date seed (*Phoenix dactylifera*) was used as a substrate.

Date seeds were scraped to remove the outer coats, and then were thoroughly washed with sand and soap to remove all reducing sugars possible. The seeds were then rinsed in distilled water, cracked, and the embryos removed. The hemicellulose thus prepared was autoclaved in distilled water at 12 pounds pressure for 20 minutes to kill all enzymes present. The water was again decanted off, and the endosperms rinsed and allowed to remain in distilled water with toluol to preserve for future use.

Van Tieghem cells were prepared, and very thin slices of hemicellulose were suspended in hanging drops of enzyme dispersion, as follows:

(1) Eight cells with hanging drops of mycelial dispersion.

(2) Eight cells with hanging drops of sporophoral dispersion.

(3) Four cells with hanging drops of autoclaved mycelial dispersion.

(4) Four cells with hanging drops of autoclaved sporophoral dispersion.

(5) Four cells with hanging drops of distilled water.

In the bottom of each cell was placed enough of the solution of the same vapor tension as the hanging drop, so that evaporation of the drops was prevented. A drop of chloroform was added to each cell as an antiseptic. These cells were examined from time to time, but no sign of the erosion of the hemicellulose was noticed until after 25 days. There was a slight indication of erosion in five of the drops of mycelial dispersion. The other three were contaminated with bacteria and showed slight erosion. There was no erosion in the controls except in one contaminated with bacteria. After 40 days three of the five drops of mycelial dispersion observed 2 weeks before were still perfectly aseptic, and two had dried down. The pieces of hemicellulose in the three cells were strongly eroded. In places only a granular substance was left. When the cover glasses were removed from these three cells there was a strong odor of chloroform still remaining in each. That they were perfectly aseptic was proven by removing from the drop what remained of the slice of hemicellulose, drying down the hanging drops on the cover slips, and flaming and staining the smear with gentian violet. There were no bacteria or fungi present. There was no erosion in any of the cells containing the enzyme preparation from the sporophores nor in the cells containing the mycelial dispersion which had been autoclaved. These results go to show that there is hemicellulase in the mycelium of *L. saepiaria* but not in the tissues of the fructifications.

Another experiment was conducted as follows: Four test-tubes each were prepared with mycelial and sporophoral dispersions in the following manner:

(1) 0.5 gm. hemicellulose+10 cc. enzyme preparation+toluol.

(2) 0.5 gm. hemicellulose+10 cc. enzyme preparation+toluol.

(3) 0.5 gm. hemicellulose+10 cc. enzyme preparation (autoclaved)+toluol.

(4) 0.5 gm. hemicellulose+10 cc. distilled water+toluol.

These were incubated at 25–30°C. for 25 days, after which the liquid was filtered off and 5 cc. from each were tested with Fehling's solution for reducing sugars. Numbers 1 and 2 showed slight reduction of copper produced by the mycelial dispersion but not by that from the sporophores. In the controls no copper oxide could be detected.

From these results it is certain that the mycelium contains the enzyme, hemicellulase, capable of hydrolyzing the hemicellulose of the endosperm of *Phoenix dactylifera.* This hemicellulose is a paragalactan, which, on hydrolysis, yields a mixture of galactose and arabinose, both of which reduce Fehling's solution.

Pectase and pectinase.—Closely allied with cellulose is a group of substances called pectic bodies. Pectose is the name given to the parent substance of bodies, such as pectin, pectic acid, etc. Many fruits, such as apples, gooseberries, currants, cranberries, and fleshy roots—such as carrots—contain a substance soluble in water but gelatinizing in alcohol. This substance which causes the juice of fruits to "jell" is known as pectin. A solution of pectin gelatinizes on standing, probably due to the action of the enzyme, pectase, contained in the fruit juice.

Mangin ('92, '93) investigated the pectose group of substances and divided them into two groups: first, neutral bodies varying in their solubility in water from pectose, which is insoluble and closely resembles cellulose, to pectin, which is soluble but readily forms a jelly; second, acid bodies, chiefly pectic acid, the latter occurring as calcium pectate, forming the middle lamellae of plant tissues. The enzyme which is capable of hydrolyzing the pectic bodies is generally termed pectinase, while the one causing coagulation is pectase.

It is a well-known phenomenon in certain types of decay that the middle lamellae disappear. As early as 1886, de Bary ('86) observed that the mycelium of *Peziza sclerotiorum* was capable of penetrating cell walls and gelatinizing them. The juice of the sclerotia of this fungus had the power to dissolve the middle lamellae and gelatinize the inner layers of the cell walls of turnips and carrots. The enzyme preparation precipitated from the juice by means of alcohol affected the cell walls in the same way.

Ward ('88) observed the macerating action of the *Botrytis* causing the lily disease. These observations were made on sections of the leaves, petioles, and ovary of the lily. The middle lamellae underwent dissolution in a few hours when placed in aqueous extracts of the fungus. Since Jones ('05) and Cooley ('14) have so amply reviewed the earlier work in this field, it will not be discussed further in this paper. Cooley ('14) shows that in tubes containing pectin a coagulum was produced by *Sclerotinia cinerea,* thus showing the excretion of pectase by this fungus, which, nevertheless, shows no particular affinity for the middle lamellae. On the other hand, Brown's ('15) work with *Botrytis cinerea* shows this fungus to possess the power of dissolving the middle lamellae. The enzyme extract prepared from very young mycelia brought about a very rapid disintegration in the tissues of potato, turnip, beet, apple, etc. Discs of these tissues were disorganized in from 15 to 90 minutes. The death of the cells did not take place until some time after they had been separated by the solution of the middle lamellae. The activity of the extract was destroyed by heat.

No work on wood-destroying forms has demonstrated the presence of an enzyme capable of disorganizing the middle lamellae, although many of the decays show maceration. Spaulding ('11) says that in the last stages of decay produced by *Lenzites saepiaria* the middle lamellae have disappeared.

Since it was found in microchemical observations that the middle lamellae of the wood decayed by *L. saepiaria* were dissolved out, it was attempted to demonstrate pectase and pectinase experimentally. Pectin was prepared in the usual way by the action of alcohol on the juice expressed from cranberries. Pectase from the carrot coagulated this pectin, but only negative results were obtained with the enzyme dispersions from *L. saepiaria.* Further experiments were conducted to determine the macerating power of the dispersions on various tissues.

Slices of carrot, potato, and beet were cut to a uniform thickness. From these slices discs were cut by means of a cork-borer, and similar discs were also prepared from very young tobacco leaves. As a source of enzymes, the mycelial powder prepared from mycelium grown on carrot juice was used. Two grams of this powder were soaked in 50 cc. of distilled water for 5 hours, after which the liquid was decanted off. The four kinds of discs mentioned above were placed in portions of this liquid in closed stenders and a few drops of toluol added to each. Discs were also kept in distilled water as controls. Observations were made after 18 hours. Carrot discs in the mycelial extract had lost in coherence in comparison with those in the distilled water. When pulled apart the latter were torn as much across the cells as following the cell walls, while in the former the separation followed the line of the cell walls. Beet discs showed no maceration whatever after 18 hours. Potato discs showed a more marked maceration than the carrot. The potato had become very flaccid in the extract. The discs of tobacco leaves showed no loss of coherence. After 42 hours the carrot discs and potato discs had lost all coherence, and the cells were easily pressed apart under a cover glass. In the controls in distilled water there was no diminution of coherence of the tissues. Thus, the presence of pectinase may be demonstrated in the mycelium of *L. saepiaria,* while there are no indications of the presence of pectase.

The effect of the cyto-hydrolyzing enzymes as demonstrated by microchemical observations on the sound and decayed wood.—Pine wood is composed of tracheids with one row of bordered pits in the radial walls. The annual rings are usually well differentiated into spring and summer wood, especially in the heart. Resin ducts occur among the tracheids, extending longitudinally, as well as radially, in the medullary rays. The resin ducts are surrounded with wood parenchyma. The sap-wood is lighter in color than the heart, probably due to oxidation, since the lumen of the heart tracheids are well aërated, as well as to the presence of tannoid bodies which become darker with continued exposure to air. The sap-wood, of course, is not so thoroughly lignified as the heart-wood, and often the inner lamella gives the cellulose test with zinc chloriodid.

The wood that has been attacked by *L. saepiaria* is darker in color than the sound wood; it is also very brittle and when crushed between the fingers breaks into a fine powder. It is evident that marked changes take place in the wood due to the action of enzymes produced by the fungus. To determine what some of these changes are and something about their sequence, I resorted to microchemical tests. To this end sections of sound wood and wood in various stages of decay were examined. Free-hand sections were made longitudinally, but in the later stages of decay it is impossible to cut transverse sections because of the brittle character of the tissues. Small pieces, carefully cut down to 0.5-cc. cubes, were imbedded in celloidin, and from these then the free-hand, transverse sections were cut.

The sound sap-wood gave the following tests:

(1) An alcoholic solution of phloroglucin with an addition of hydrochloric acid gave a deep red in the middle lamella, dark red in the secondary, and pink in the tertiary lamella.

(2) After soaking sections in iodine and then treating with 65 per cent sulphuric acid, the secondary lamella was a yellow to brown color, with a purple lining in the early spring wood.

(3) Chloriodid of zinc gave a brown coloration both in the spring and summer wood, with a slight blue tint lining the tracheids in the sap-wood.

(4) Aniline sulphate produced a bright yellow which increased in intensity from spring to late summer wood.

(5) After treating with potassium hydroxide for some time and then applying the cellulose tests the following results were obtained:

(a) After continued action of iodine followed by the addition of sulphuric acid, the inner or tertiary lamella was swollen and shrunken away from the secondary, the former assuming a purple color.

(b) Chloriodid of zinc colored the swollen tertiary lamella blue, while the main part of the wall was brown.

(6) Resorcin and sulphuric acid gave a violet to blue reaction in the lignified walls.

When these same tests were applied to the decayed wood some difficulty was found, especially where the tests yield yellow or brown, because the tissues were decidedly brown in the last stages of decay. If a transverse section is made through a decayed portion of wood so as to include a part of the sound, normal wood, the progressive stages of decay may be followed by applying the above stains.

When phloroglucin and hydrochloric acid are applied to such a section, it is noticed that chemical changes have preceded any visible or microscopical changes in structure. In the sound wood the secondary and tertiary lamellae are stained a dark red, while the middle lamella is a still darker red. A little nearer the edge of the decayed region the red has changed to a maroon or brownish red in the tertiary lamella. As we proceed nearer to the decayed portion this maroon increases until the secondary lamella is all brownish red, and the middle lamella only remains the brighter red with this stain. In radial sections it may be noticed that this maroon discoloration starts from the bordered pits, especially if they are perforated with fungous hyphae. The hyphae seek the bordered pits, these apparently serving as the only places where the hyphae pass from one lumen to another.

In the tangential section the discoloration in the earliest stages occurs only in the neighborhood of the medullary rays. While this discoloration is taking place the tertiary lamella first contracts and then practically disappears. The secondary lamella likewise shrinks as it takes on this brownish red color. This must be due to the gradual hydrolysis of cellulose and probably the simultaneous hydrolysis of coniferin through the action of emulsin. The secondary lamella shrinks, but in the last stages of decay there are still brown, fragile remains of this layer, together, undoubtedly, with infiltrated by-products from the decayed middle and tertiary lamellae. The middle lamella seems to disappear almost simultaneously with the decay of the secondary.

With chloriodid of zinc the decayed wood gives the same test as in the sound wood, a brown color. This is true in all stages of decay. There is no indication of free cellulose at any time during decay. Partially delignified sections, cut from the same surface as the sections on which the above lignin tests were made, were treated with a 5 per cent potassium hydroxide for some time. On the addition of chloriodid of zinc the lamellae yielded a purplish blue reaction, where only partially delignified by the fungus; but where decay was complete a brown color was obtained. These results show that the first step is the splitting of lignin, and simultaneously with this there is a complete hydrolysis of the cellulose as fast as it is set free. Undoubtedly some of the substances giving the lignin reaction are also used up.

Other lignin tests gave similar results. The action of aniline sulphate was marked. The sound wood was colored yellow, and as the diseased region was approached the color became browner, although the yellow element did not seem to be lost entirely. Indications are that in the decayed wood some of the substance that gave the lignin reaction still remained. This was easily extracted and is what Czapek has called "hadromal." The iodine-sulphuric acid test for cellulose corresponds well with what we found with zinc chloriodid —a light brown color.

Our microchemical tests applied to the decayed wood substantiate in the main the results obtained with the enzyme dispersions and other enzyme preparations, i.e., that cellulase and ligninase are secreted by *L. saepiaria.* Pectinase is both demonstrable *in vitro* and in nature, the pectinase of the middle lamella disappearing with the action of the fungus.

A point of further interest is the composition of the brown substance left after the complete decay of the tracheids. This is a brittle substance which is easily crushed into a fine brown powder. A quantity of this brown material secured from the decayed cavities of an old railroad tie was ground as finely as possible in a mill, and to this powder was added a dilute alkali. After soaking for 2 days the alkali was filtered off, and by adding acid to the filtrate a flocculent precipitate was thrown down. When dried down this precipitate shrinks and cracks. It is insoluble in chloroform, alcohol, ether, acetone, and petroleum ether, but is readily redissolved in alkali and may be reprecipitated with acid. This substance partakes of the nature of "humus" compounds. The remainder of the brown powder is much like "peat."

In the disease of *Taxodium distichum* known as "pecky" cypress, von Schrenk ('00[b]) found a similar substance which he called a humus compound. In the case of *Taxodium* the humus compounds are in a liquid form, and thus are deposited in the tracheids where the mass dries and cracks, "looking much like mud which has dried in the sun." The humus liquid infiltrates into the sound wood immediately surrounding the decayed spots and darkens the wood in the decayed regions. Undoubtedly, the humus compounds found in wood decayed by *Lenzites* and by the fungus causing the peckiness of cypress are a direct result of the activity of the enzymes concerned in the decay.

It was mentioned above that in the process of delignification, etc., the shell of the tracheids remaining after the last stages of decay shrinks and cracks. The cracks in the walls always follow the same general direction and seem to begin with the bordered pits. The slits are spiral in form and pass obliquely across the pits from left to right upwards. By changing the focus these lines are from right to left upwards on the farther wall of the tracheid. Von Schrenk ('00^b) also observed this in the tracheids of *Taxodium distichum* infected with "peckiness." In the erosion of the cell walls of Elodea leaves, due to the action of an enzyme preparation from *Merulius lacrymans,* Kohnstamm ('01) noticed that the action took place in definite lines. He is inclined to believe that this is due to the micellar structure of the cell walls.

In general, the Lenzites decay is of the same type as that produced by *Merulius lacrymans* and reported on by Czapek ('99), but the hydrolysis of the cellulose is much more rapid in the former. In contrast to this type we have the other extreme represented by the pin rot of pine due to *Trametes Pini,* the white rot of cedar due to *Polyporus juniperinus,* and the rot of oak produced by *Thelephora perdix.* In this case, as mentioned before, all substances are used by the fungus with the exception of cellulose which is left as a pure white by-product.

INULINASE

Inulinase was discovered by Green ('88) in the Jerusalem artichoke (*Helianthus tuberosus*). It was first demonstrated in fungi by Bourquelot ('93) who found it in *Aspergillus niger,* but later with Hérissey ('95) did not find it in the sporophores of *Polyporus sulphureus.* Dean ('03) verified Bourquelot's work with *Aspergillus niger* and *Penicillium glaucum* and found inulinase to be an intracellular enzyme. Dox ('10) reported that *Penicillium Camemberti* had slight action on inulin unless cultivated on a substrate containing inulin as the source of carbon. In this case inulinase was produced more abundantly.

In our experiments with *Lenzites* the enzyme preparations from the mycelium and the sporophores were used. To 10 cc. of a 1 per cent solution of inulin 2 cc. of the enzyme dispersion were added and toluol used as an antiseptic. There was a marked reduction of copper oxide from Fehling's solution after 2 days of incubation at 25–30° C., while the boiled controls and water controls showed no reduction. The inulinase seemed to be quite active in both the mycelium and sporophores, but no quantitative comparison was made.

AMIDASE AND UREASE

Since we found positive proof of the presence of tryptic and ereptic ferments in the fungus, the next logical step in sequence was to ascertain whether amidases were present, for protein digestion normally proceeds further than to the peptone stage and results in the amino acids, which, digested by amidases, yield ammonia and hydroxy-acids.

In the lower fungi these desamidizing enzymes have been found by many workers. Butkewitsch ('03) found by growing cultures of *Aspergillus, Penicillium,* and certain species of *Mucor* on liquid media, containing proteins that ammonia is liberated; and in the following year Shibata ('04) found in a "Dauerpräparat" of the mycelium of *Aspergillus niger* an enzyme which split ammonia from different nitrogen-containing substances, while Pringsheim ('08) found the same enzyme present in "Acetonedauerhefe." Dox ('10) found that the enzyme preparation from *Aspergillus niger* and *Penicillium Camemberti* showed the power to split ammonia from asparagin and urea. Reed ('13) found very similar results by the action of the enzyme powder from *Glomerella rufomaculans* on asparagin and alanin. The only record of urease from one of the higher fungi was that reported by Kikkoji ('07) in *Cortinellus edodes.*

In the experiments reported in the following tables the enzyme dispersions from both the mycelium and sporophores and also the fungous powder from both tissues were used. As substrates 50 cc. of 1 per cent solutions of asparagin, acetamid, and urea were used. Ten cubic-centimeter portions of the enzyme dispersions were used in some cases, while an equivalent weight of the fungous meal was used where the enzymes were not extracted and precipitated. Autoclaved controls, as well as the substrates alone, were set up for each series, and toluol was used as an antiseptic in all cases. Each experiment was set up in a gas-washing bottle, which was fitted with rubber tubes securely stoppered. These washing bottles were incubated at 25–30°C. for 20 days, when the ammonia was determined by the Folin method. Friedrich's improved gas-washing bottles containing 250 cc. of N/50 HCl were used for the collection of the ammonia. Air was drawn through for 1½ hours by means of a Richards' suction pump,

TABLE XII

AMIDASE ACTION IN THE MYCELIUM OF LENZITES SAEPIARIA

Substrate	Form of enzyme material	Nitrogen as ammonia set free	Net nitrogen set free
		mg.	mg.
Urea	Enzyme dispersion	2.10	0.28
Urea	Enzyme dispersion (autoclaved)	1.82	....
Urea alone	....	1.12	....
Urea	Meal	23.55	22.57
Urea	Meal (autoclaved)	0.98	....
Asparagin	Enzyme dispersion	1.40	0.00
Asparagin	Enzyme dispersion (autoclaved)	1.04	....
Asparagin alone	....	0.14	....
Asparagin	Meal	0.21	0.07
Asparagin	Meal (autoclaved)	0.00	....
Acetamid	Enzyme dispersion	1.12	0.00
Acetamid	Enzyme dispersion (autoclaved)	1.12	....
Acetamid	Meal	0.14	0.14
Acetamid	Meal (autoclaved)	0.00	....
Acetamid alone	....	0.00	....

TABLE XIII

AMIDASE ACTION IN THE SPOROPHORAL TISSUE OF LENZITES SAEPIARIA

Substrate	Form of enzyme material	Nitrogen as ammonia set free	Net nitrogen set free
		mg.	mg.
Urea	Enzyme dispersion	5.46	—
Urea	Enzyme dispersion (autoclaved)	6.16	....
Urea alone	....	6.16	....
Urea	Meal	18.34	12.18
Urea	Meal (autoclaved)	5.88	....
Asparagin	Enzyme dispersion	1.89	0.91
Asparagin	Enzyme dispersion (autoclaved)	0.98	....
Asparagin	Meal	2.24	1.12
Asparagin	Meal (autoclaved)	1.12	....
Acetamid	Enzyme dispersion	2.03	1.75
Acetamid	Enzyme dispersion (autoclaved)	0.28	....
Acetamid	Meal	0.28	—
Acetamid	Meal (autoclaved)	0.84	....

then duplicate portions of the collection acid were titrated against N/50 NaOH. Alizarin red was used as an indicator. Tables XII and XIII show the results obtained.

It is interesting to notice that urease is the only active enzyme in the fungus which produces desamidation, and that this enzyme was not extracted with water. Pringsheim ('08) has shown that the amidases of yeast do not pass out with the water extract but are tenaciously held by the protoplasm. The same may be the case with urease of *Lenzites,* and since we assume that the enzymes are protein-like bodies it is possible that urease in this case is not like the albumins, which are water-soluble. It may be a globulin, soluble in neutral saline solutions, or glutelin, soluble in weak alkali.

It is again of interest here to notice that the enzyme is much more active in the mycelial than in the sporophoral tissue, even without considering the amount of sawdust present in the mycelial meal. Of course, here, as in other plants, it is not possible to connect the presence of urease with any useful function in the metabolism of the fungus. With our present knowledge of desamidation in plants the biological importance of urease remains unknown.

HIPPURICASE

The ability of the lower fungi to bring about the splitting of hippuric acid by enzyme action was first noticed by Shibata ('04) in the case of *Aspergillus niger.* Some years later Dox ('09) demonstrated the presence of hippuricase in other species of fungi, namely, *Penicillium Camemberti, P. chrysogenum,* and *P. brevicaule,* and he also confirmed Shibata's results on *Aspergillus niger.* The enzyme preparation was the ground fungous mycelium after it had been dried by the usual "Acetondauerhefe" method. His method of determining the amount of hippuric acid hydrolyzed is given in a later paper (Dox, '10). After hydrolysis by the enzyme the hippuric acid solution, which was made up in weak sodium hydroxide, was mixed with the calculated amount of sulphuric acid to combine with the sodium. This was shaken with petroleum ether which dissolves out the benzoic acid. The latter crystallizes when the petroleum ether is evaporated, and the crystals, after recrystallization from water, melted at 121°C. and had the appearance of benzoic acid. In the controls no residue was obtained. The hydrolysis in the case of *Penicillium Camemberti* was 76 per cent.

In 1912 Kossowicz found that enzyme preparations of *Aspergillus niger, Mucor Boidin, Phytophthora infestans, Isaria farinosa, Botrytis Bassiana,* and *Cladosporium herbarum* in every case brought about the destruction of hippuric acid. As one of the reaction by-products he identified ammonia, but in no case did he state in how great quantities the ammonia was formed. Reed ('13) found that an enzyme powder prepared from *Glomerella rufomaculans* showed the presence of hippuricase after incubation for one week.

In 1913 Dox and Neidig applied Sörensen's formaldehyde-titration method for the determination of the acidity of amino acids to the hippuric acid hydrolysis. The method is founded on the reaction between formaldehyde and the primary amino group. Hippuric acid has no primary amino group, but after hydrolysis the primary amino group of the glycocoll split off may be neutralized with formaldehyde, leaving the carboxyl unchanged to be titrated against an alkali. In this case Dox and Neidig used N/10 $Ba(OH)_2$. They grew cultures of *Aspergillus niger, A. clavatus, A. fumigatus, Penicillium expansum, P. Roqueforti,* and *P. Camemberti* on a nutrient solution. The cultures were grown for 1, 2, 3, and 4 weeks, the juice pressed out after grinding the mycelium and used as an enzyme preparation. In all of these fungi hippuricase was found, as well as in taka-diastase (*Aspergillus Oryzae*). The age of the mycelium had little influence on the production of hippuricase. Titrations for free ammonia showed that in the cultures 3 and 4 weeks old there were slight amounts, if any, of ammonia, and a secondary reaction, or the splitting of glycocoll, is improbable.

Our experiments with the meal from the mycelial and sporophoral tissues of *Lenzites* were carried out in the same way as those described by Dox ('10). Fifty cubic-centimeter quantities of the substrate were used, and the flasks were incubated at 25°C. for 2 weeks. After this period the benzoic acid was dissolved out with petroleum ether, dried, and then recrystallized from water. From 50 cc. of a 1 per cent solution of hippuric acid were obtained 0.24 grams of benzoic acid, or there was 65 per cent hydrolysis. The melting point was found to be 126°C. Since the melting point of hippuric acid is 187°C. and that of benzoic acid is 121°C., this was regarded as evidence that the crystals were benzoic acid.

NUCLEASE

The presence of nuclease has been demonstrated in various divisions of the plant kingdom. It performs an important function in decomposing the nucleic acids of plant cells, especially in germinating seeds or wherever dissimilation is carried on. In the fungi nuclease has been found both in the lower and higher forms. Iwanoff ('03) studied the effect of cultures and enzyme extracts of *Aspergillus niger* and *Penicillium glaucum* on nucleic acid, and observed that both species produced the purin bases and phosphoric acid in the substrate. He claims that the nuclease is distinct from the proteolytic enzymes, for his enzyme extract would not liquefy gelatin. Dox ('10) found that the nuclease of *Penicillium Camemberti* is formed irrespective of the presence of nucleic acid in the culture medium. Kikkoji ('07) expressed the juice from an agaric, *Cortinellus edodes,* and 25 cc. of this in 150 cc. of a 2⅔ per cent solution of the sodium salt of nucleic acid produced 28.7 mg. of phosphorus pentoxide in 5 days' digestion. He considered this action due to nuclease, the juice being thermolabile.

In my work with *Lenzites saepiaria* a 1 per cent solution of phyto-nuclein from yeast was prepared by dissolving the phyto-nuclein in N/20 sodium hydroxide and then neutralizing. To 25 cc. of this solution 5 cc. of the enzyme dispersion were added with toluol as an antiseptic. Controls were set up by adding 5 cc. of autoclaved dispersion to the nuclein solution, and water controls made by adding 5 cc. of distilled water to the nuclein. This plan was followed both for mycelial and sporophoral dispersions. The flasks were incubated at

25–30°C. for 21 days, after which they were titrated for phosphoric acid which was calculated as phosphorus pentoxide according to the uranium acetate method described by Hawk ('12, p. 413). One cubic centimeter of the solution of uranium acetate used was calculated to be equivalent to 4.65 mg. of phosphorus pentoxide. Two cubic centimeters of a solution of sodium acetate were added to 10 cc. of the filtered substrate to be titrated. This was brought to a boil and titrated while hot. Potassium ferrocyanide was used as an indicator. Table XIV shows the results of these experiments, the figures given being the averages of duplicate experiments which were run in all cases.

TABLE XIV

NUCLEASE ACTIVITY IN LENZITES SAEPIARIA

Amount of enzyme dispersion used with 25 cc. of 1 per cent nuclein	Free H_3PO_4 as P_2O_5 after 21 days		Net P_2O_5 in 25 cc. of substrate	
	Mycelium	Sporophore	Mycelium	Sporophore
	mg.	mg.	mg	mg.
5 cc. enzyme dispersion	40.11	59.31	28.49	37.83
5 cc. enzyme dispersion (autoclaved)	11.62	22.08	......	....
5 cc. distilled water	9.89	....	......	....

These results demonstrate the presence of nuclease both in the mycelial and sporophoral tissues of *Lenzites saepiaria*. However, no quantitative comparison can be drawn, for the mycelial dispersion was made from the mycelial mat in sawdust. Compared with the results obtained by Kikkoji, the sporophoral dispersion is not so active as that in *Cortinellus*. This may be due to the fact that he used a more concentrated substrate and the juice of the fresh fungus direct.

The fact that phyto-nuclein, an alpha-nucleoprotein, was used as a substrate, and that this was broken down to phosphoric acid is a definite control on our results illustrating tryptic enzymes. The nucleoprotein must first be broken down by a nucleinase, which, according to the consensus of opinion, is a tryptic ferment. It is only after this enzyme has acted that the nucleic acid is free to be acted upon by nuclease.

PROTEASE

Proteolytic enzymes have been commonly demonstrated in the filamentous fungi, especially *Aspergillus* and *Penicillium,* but in the higher forms they are not so well known. The first to discover protein-digesting enzymes in the *Basidiomycetes* were Bourquelot and Hérissey ('95) who found that pieces of the white of an egg, which had been cooked for 10 minutes on a water bath, were changed when placed in the juice expressed from sporophores of *Polyporus sulphureus.* The solution gave a slight biuret reaction after incubating at 29–30°C. for 21 hours. Hjort ('96) found in the sap from the sporophores of *Pleurotus ostreatus* a tryptic ferment capable of digesting fibrin. It worked best in acid solutions and produced leucin, tyrosin, and tryptophan. The naturally acid watery extract of the sporophores of *P. sulphureus* readily digested fibrin, but if neutralized or made alkaline it did not act at all. The expressed juice, weakly acidified with hydrochloric acid or oxalic acid, digested fibrin as well as the original extract alone. After 12 hours of digestion the action was carried to peptones only. There were no amino acids present.

In 1898 Bourquelot and Hérissey investigated the same action of the expressed juice of the sporophores of *Amanita muscaria,* and found it to digest nearly all of the caseinogen of skimmed milk in 4 days, after which tyrosin was present in the solution. Kohnstamm ('01) investigated the proteases of *Armillaria mellea, Merulius lacrymans,* and *Polyporus squamosus.* The expressed juice from the sporophores of *Armillaria mellea* liquefied neutral thymol-gelatin to the extent of 1 mm. in depth in 10 days. The gelatin tubes were 8 mm. in diameter. Both the mycelium and sporophores of *Merulius lacrymans* were used. The juice from both was equally active in liquefying gelatin, about 8 mm. in 10 days or equivalent to 0.6 cc. The active principle is thermolabile. In a 0.2 per cent solution of hydrochloric acid the extract digested fibrin to peptone but not to amino acids, while in 0.2 per cent sodium carbonate solution there was no digestion of fibrin. The juice of sporophores of *P. squamosus,* collected in January and March, liquefied gelatin at the rate of 1 mm. per day for 30 days. There was the same action on fibrin as related above for *Merulius lacrymans.*

Vines ('03) found by allowing crushed sporophoral tissue of *Agaricus campestris* to act on fibrin for 22 hours that there was a complete digestion to amino acids, i.e., the tissue is able to peptonize fibrin and digest the peptones. Delezenne and Mouton ('03, '03^a), a little later in the same year, secured widely different results. From the dried fruiting bodies of *Agaricus campestris, Amanita muscaria, A. citrina,* and *Hypholoma fasciculare* they made extracts with 0.8 per cent sodium chloride, using chloroform or toluol as antiseptics. The extracts thus prepared from all of these species converted peptone to amino acids, digested gelatin and casein, but would not peptonize fibrin. These results of Delezenne and Mouton seemed so contradictory to the observations of previous workers that Vines ('04) made further experiments to test their accuracy.

For this work the ground pulp of the sporophores of *Agaricus campestris,* with the lamellae removed, was used. Providing the sporophores were mature the digestion of fibrin was evident. However, when a watery extract of the pilei was used the results were less certain, but in other experiments Vines found that extracts made with 2 per cent sodium chloride from fresh and dried sporophores actively digested fibrin in 1 per cent toluol or 0.2 per cent hydrocyanic acid. Since he found that boiled fibrin was not digested by these extracts, he suggests that the negative results of Delezenne and Mouton must be due to this error. Thus the extracts of *A. campestris,* prepared by Vines, contained an enzyme capable of peptonizing fibrin and converting the peptones and albumoses to amino acids. From this he concludes that there are two distinct proteases present, the one trypsin, the other erepsin.

Buller, in 1906, confirmed the results obtained by Kohnstamm in 1901 on the presence of proteases in the sporophores of *P. squamosus.* Kikkoji ('07) demonstrated the presence of a protease in the sporophores of *Cortinellus edodes.* It acted in neutral or alkaline solutions.

Rumbold ('08), in cultural studies on various wood-destroying fungi, investigated the action of 13 of them on gelatin of the following constitution: 2½ per cent Liebig's beef extract; 2½ per cent malt extract; 10 per cent gelatin. The reaction was adjusted in one lot by use of sodium carbonate and in another by the use of sodium hydroxide. She found that *L. saepiaria* was the only one to liquefy gelatin, and this only when sodium carbonate was used to readjust the reaction of the medium.

Experiments were conducted using various substrates such as gelatin, albumin, casein, legumin, peptone, and fibrin. The enzyme preparations and fungous meal were used in some cases, while in others the growing fungus was utilized.

Action on gelatin.—A 10 per cent gelatin was prepared in the same way as that used by Rumbold ('08), cited above. The reaction was adjusted with sodium carbonate and sodium hydroxide, and poured plates prepared and inoculated with mycelium of *Lenzites.* After a growth of 4 days a circle of gelatin 1.2 cm. in diameter was liquefied in the region of the inoculum, but this only in those plates neutralized with sodium carbonate, while there was no liquefaction in those neutralized with sodium hydroxide. In the latter the mycelium had penetrated the gelatin to some extent. Gelatin in the form of Mett's tubes was digested by the enzyme dispersion of the mycelium, but sodium hydroxide did not show the same inhibiting effect as mentioned above. Negative results were obtained with the sporophoral dispersion. These results are quite in accord with experiments of other workers who have investigated the influence of the reaction of the medium on the growth of timber-destroying fungi. In this case the results suggest that there is an effect of alkalis on the metabolism of the fungus, i. e., that the secretion of proteolytic enzymes by the living organism is checked by the toxic effect of such active alkalis as sodium hydroxide, but when once excreted they have no inhibitory effect on the enzyme action.

Action on albumin, casein, legumin, and peptone.—The proteolytic action of the enzymes of the sporophoral and mycelial dispersions was tested by means of various substrates, under acid, alkaline, and neutral conditions. One per cent solutions of albumin, casein as such, as well as in the form of commercial "nutrose," legumin, and peptone were prepared. Casein and legumin were dissolved in N/10 NaOH. Albumin was also used in the form of Mett's tubes.

To determine the tryptic action the biuret test was employed. In testing for peptone by the biuret test it is necessary to get rid of the native proteins, since these give a purplish blue coloration with alkaline copper sulphate, the purple blinding the fainter pink test for peptone. In order to do this ammonium sulphate is usually added in crystalline form to precipitate the higher proteins. The precipitate, however, is generally in such finely divided particles that it will not filter out with the filter papers commonly used. To overcome this difficulty the solution was filtered through bone black to remove the precipitate. The question arose whether the bone black might not absorb the peptone, and tests were made as follows: To a few cubic centimeters of 1 per cent casein solution was added a small bit of Witte peptone. Crystals of ammonium sulphate were added to precipitate the casein. After filtering through bone black the clear filtrate gave a pink color with sodium hydroxide and dilute copper sulphate.

The tryptophan test was employed in testing for the action of erepsin. Wherever a test for tryptophan was given, higher proteins being used as a substrate, there was a demonstration of tryptic action as well. The tryptophan test is the production of a pink color after the addition of a few drops of glacial acetic acid and then a few drops of strong chlorine water.

Experiments were set up using 10-cc. portions of the abovementioned substrates in tubes. Two cubic centimeters of the enzyme dispersions were added to each tube, except the water controls. Toluol was used as an antiseptic. Each substrate was set up in series of neutral, acid, and alkaline cultures, the acidity and alkalinity being approximately N/200.

Where casein, legumin, and albumin were used as substrates there was no indication of digestion in either the mycelial or sporophoral dispersion. In no case was there positive proof of peptonization. Peptone, however, was peptolyzed in neutral, alkaline, and acid solutions. If any distinction can be made there was stronger indication of tryptophan in the acid and alkaline solutions than in neutral. Quantitative determinations were not made.

Action on fibrin.—The digestion of fibrin was determined by using the method described by Reed ('13). Fibrin was stained in 1 per cent Congo red, and the color fixed by immersion in hot water. When such fibrin is acted on by trypsin the red color is liberated into the solution. With this colored fibrin in water as a substrate, negative results were obtained with the mycelial and sporophoral dispersions. Toluol was used as an antiseptic. Another series of tubes was set up, using 6 grams of mycelial meal in 10 cc. of water and the colored fibrin as a substrate. In this case potassium cyanide was used as an antiseptic, and the results were positive. The color value in this case was difficult to judge, however, on account of the brown color imparted to the solution by the sawdust meal, but the tryptophan test confirmed the color liberation.

The question arose whether potassium cyanide is a better antiseptic than toluol, use of the first-mentioned having been recommended by Vines in certain cases. Later experiments were conducted, using the pure mycelium grown on carrot juice as a source of enzymes, while potassium cyanide, toluol, and thymol-chloroform were used as antiseptics. In this series pure fibrin in water was the substrate. In all of these except two, after digestion of two weeks, the tryptophan test was obtained, the exceptions being those of the autoclaved controls and water controls. The test was slightly more distinct where potassium cyanide was used as an antiseptic.

The results are sufficient to demonstrate the presence of both erepsin and trypsin in the mycelium, and at least erepsin in the sporophores of *L. saepiaria*.

RENNET

The property possessed by the juice of certain plants of causing milk to coagulate was known as early as the sixteenth century, notably in the case of *Galium verum.* According to Green ('93), this plant is still in use at the present day for the coagulation of milk in cheese-making. Green, Oppenheimer ('10), and Euler ('12) have comprehensively reviewed the literature on the occurrence of rennet in the higher plants. Oppenheimer ('10, p. 317) also lists certain bacteria, such as *Bacillus amylobacter, B. mesentericus* var. *vulgatus,* and *B. prodigiosus,* which cause the coagulation of milk. It has been observed by a few workers that rennet is present in certain fungi, especially *Aspergillus* and *Penicillium* (Oppenheimer, p. 317). Buller ('06) found this enzyme to be very active in the juice from the sporophores of *Polyporus squamosus,* and Bayliss ('08) reports it for *Polystictus versicolor.* In fact, the existence of rennet in the *Basidiomycetes* is considered general. Gerber ('09) has demonstrated its presence in 86 species. Of the wood-destroying forms, he examined *Stereum purpureum* Fr., *Polyporus adustus* Willd., *P. betulinus* Bull., *P. hispidus* Bull., *P. giganteus* Pers., *Trametes Bulliardi* Fr., *T. gibbosa* Pers., *T. suaveolens* L., *Daedalea borealis* Wahlb., *Hydnum repandum* L., *Armillaria mellea* Vahl, and *Lycoperdon piriforme* Schaeff.

Our experiments show that rennet is present in both the mycelium and sporophores of *L. saepiaria.* After incubating at 25–30°C. for 3 hours the coagulum was formed by the mycelial dispersion, but it took 7 hours to form a coagulum in the sporophoral dispersion. There was no coagulation in the boiled or water control after 20 hours, the toluol keeping it antiseptic. The results show that the rennet is more active in the mycelium than in the sporophores.

The biological importance of this enzyme in plants is entirely a mystery. There arises the supposition *a priori* that one has to do here with some peculiar phase of protease activity, since in animal life the activity of rennin seems to be closely linked with the proteases of the gastric and pancreatic juices.

OXIDASE AND CATALASE

Interest was first aroused concerning oxidases in higher fungi because of the discoloration of certain fungous tissues when exposed to the air. During the years 1895, '96, and '97 many papers appeared by Bourquelot, Bertrand, Hérissey, et al., dealing with the oxidases in the higher fungi. They found that laccase was widely distributed in the *Basidiomycetes,* and in the case of *Boletus cyanescens,* the bluing of injured spots was due to the laccase acting with the oxygen of the air on the boletol present in the tissues.

Bertrand ('96) showed that the crystalline chromogen in *Russula,* especially *Russula nigricans,* was tyrosin, and in the latter the tyrosin on exposure to the air was oxidized to melanin, a black substance. Tyrosinase in the tissues oxidized the tyrosin, causing the tissues to blacken.

Lutz ('12), investigating the oxidases in the stipes and pilei of *Gyromitra gigas* and *Disciotis perlata,* found tyrosinase present in both species, but in both a more marked action in the caps than in the stipes. Euler ('08) carefully investigated the catalase of *Boletus scaber.* There seemed to be a relation between the oil content of the fungus and the amount of catalase present, and the presence of a metal, like magnesium hydroxide, in the solution increased the catalytic action.

The literature concerning the function of oxidases in plants has been amply considered by Clark ('11), who makes special mention of the relation of oxidases to chromogens in the higher fungi and their possible aid in the respiration process.

In my experimental work on oxidases and catalase no quantitative determinations were made. In some instances the enzyme dispersions were used, in others the fresh tissues. Clark's ('10) methods were used, and guaiacum, alpha-naphthol, and paraphenylenediamine were used as indicators of oxidation. The mycelial extract was made from pure cultures of *L. saepiaria* grown on Thaxter's potato-hard agar. Ten grams of fresh fungous mat were ground with sand and treated with 100 cc. of distilled water. This extract (5 drops in 5 cc. of H_2O_2) caused a rapid evolution of gas, showing the presence of catalase. The sporophoral meal showed much greater activity than that of the mycelium. Some sporophoral enzyme dispersion, added to hydrogen peroxide in an evaporating dish, showed active evolution of oxygen, but the mycelial dispersion treated in the same way gave none.

Oxidase action was shown by use of guaiacum and paraphenylenediamine but not with alpha-naphthol. The experiments were set up in the following way, and each series in duplicate: To 5 cc. of the fresh fungous extract were added 5 drops of hydrogen peroxide and 10 drops of the indicator. With the guaiacum a faint blue tinge was produced in 2 hours when the mycelial extract was used. When an extract from the dried sporophores was used in the same way the blue color was more distinct in 2 hours. The paraphenylenediamine gave a brownish color in about 6 hours both in the sporophoral and mycelial extracts.

Tyrosinase was demonstrated both in the sporophores and mycelium. The substrate used was a suspension of tyrosin in distilled water. To 10 cc. of this suspension 2 cc. of enzyme dispersion were added. There was no oxidation in the autoclaved controls, but in 16 hours the suspension containing mycelial dispersion had become a light gray, while that containing the sporophoral dispersion was a dark gray. Tyrosin when oxidized becomes black, but here where only partially oxidized it shows a gray color. The tyrosinase is more active in the fruiting bodies, as in the case of catalase and other oxidases.

Summary

In this paper there are considered some of the more important aspects of the physiology of a wood-destroying organism, *Lenzites saepiaria*.

1. The fungus was grown in pure cultures, and the characteristics of the mycelium and sporophores produced under cultural conditions are described.

2. Some factors influencing the growth and metabolism of the organism are discussed, and experimental results are given on the relations of the fungus to reaction of media, to water, and to oxygen. Special interest is attached to the in-

fluence of the resin content of the substratum on the growth of the fungus. A resin agar emulsion was prepared, and experimental data show that *L. saepiaria* will grow well on 50 per cent resin by weight, which is considerably more than is found in any coniferous wood. Growth is not entirely inhibited by 85 per cent resin.

3. The metabolism of the fungus was studied through the agency of enzyme action.

a. A standard method of extracting and isolating the enzymes was used, and enzyme preparations were made from vegetative and fruiting tissues. The methods commonly used of identifying the enzymes were employed.

b. Among the *esterases,* those acting on the esters of the lower fatty acids showed more active hydrolysis both in sporophoral and mycelial tissues than those acting on the neutral fats.

c. In the *carbohydrases,* positive evidence was obtained of the presence of *maltase, invertase, raffinase, emulsin, tannase, diastase, inulinase, ligninase, cellulase, hemicellulase,* and *pectinase,* while negative results were obtained for the presence of *pectase* and *lactase.*

d. The *cyto-hydrolyzing carbohydrases* were made a special study, together with their effects as demonstrated by microchemical observations on sound and decayed wood.

e. The action of *amidase* on asparagin and acetamid was practically negligible, while *urease* action was very decided when the fungous tissue was used instead of the enzyme preparations. The presence of *hippuricase* was also demonstrated.

f. The following additional enzymes have also been found: *nuclease, proteinases*—both tryptic and ereptic—*rennetase, oxidase,* and *catalase.*

4. A comparative study of the enzymes occurring in the sporophoral and mycelial tissues was made. As we would anticipate, this comparison shows that the important metabolic processes are carried on in the vegetative organs. In-

deed, wherever quantitative results were obtained, or where a comparison can be accurately made, as in the case of *diastase, invertase, tannase,* and *cellulase,* the greater activity is shown in the mycelium. An exception to this, however, is the *oxidases,* where the greater activity is in the sporophores.

ACKNOWLEDGMENTS

The writer acknowledges his indebtedness to the Southern Pine Association, who through their appropriation of funds made this investigation possible, to the Missouri Botanical Garden for library and laboratory facilities, and to Professor B. M. Duggar and Dr. Hermann von Schrenk for their advice and helpful criticisms.

BIBLIOGRAPHY

Appel, O. ('15). The relations between scientific botany and phytopathology. Ann. Mo. Bot. Gard. **2**: 275–285. 1915.

de Bary, A. ('86). Ueber einige Sclerotinien und Sclerotien-krankheiten. Bot. Zeit. **44**: 377–387, 393–404, 409–426, 433–441, 449–461, 465–474. 1886.

Bayliss, Jessie S. ('08). The biology of Polystictus versicolor (Fries). Jour. Econ. Biol. **3**: 1–24. *pl. 1–2.* 1908.

Bernthsen, A. ('12). A textbook of organic chemistry. pp. 1–720. 1912. [Edited and revised by J. J. Sudborough.]

Bertrand, G. ('96). Sur une nouvelle oxydase, ou ferment soluble oxydant, d'origine végétale. Compt. rend. Acad. Paris **122**: 1215–1217. 1896.

————, ('96a). Sur la présence simultanée de la laccase et de la tyrosinase dans la suc de quelques champignons. *Ibid.* **123**: 463–465. 1896.

Biffen, R. H. ('99). A fat-destroying fungus. Ann. Bot. **13**: 363–376. *pl. 1.* 1899.

————, ('01). On the biology of Bulgaria polymorpha Wett. *Ibid.* **15**: 119–134. *pl.* 7. 1901.

Bloor, W. R. ('14). A method for the determination of fat in small amounts in blood. Jour. Biol. Chem. **17**: 377–384. 1914.

Bourquelot, E. ('93). Les ferments solubles de l'Aspergillus niger. Soc. Myc. Fr., Bul. **9**: 230–238. 1893.

————, ('94). Présence d'un ferment analogue à l'emulsine dans les champignons et en particulier dans ceux qui sont parasites des arbres ou vivent sur le bois. *Ibid.* **10**: 49–54. 1894.

————, ('96). Des composes oxydables sous l'influence du ferment oxydant des champignons. Compt. rend. Acad. Paris **123**: 315–317. 1896.

————, ('96a). Action du ferment soluble oxydant des champignons sur les phénols insolubles dans l'eau. *Ibid.* **123**: 423–425. 1896.

————, ('97). Sur la présence générale, dans les champignons, d'un ferment oxydant agissant sur la tyrosine; sur le mécanisme de la coloration du chapeau de ces végétaux. Soc. Myc. Fr., Bul. **13**: 65–72. 1897.

———, et Bertrand, G. ('96). Les ferments oxydants dans les champignons. *Ibid.* **12**: 18–26. 1896.

———, ———, ('96a). Sur la coloration des tissus et du suc de certains champignons au contact de l'air. *Ibid.* **12**: 27–32. 1896.

———, et Harley, V. ('96). Sur la recherche et la présence de la tyrosine dans quelques champignons. *Ibid.* **12**: 153–156. 1896.

———, et Hérissey, H. ('95). Les ferments solubles du Polyporus sulfureus (Bull.). *Ibid.* **11**: 235–239. 1895.

———, ———, ('98). Recherche et présence d'un ferment soluble protéo-hydrolytique dans les champignons. Compt. rend. Soc. Biol. X. **5**: 972–974. 1898.

———, et Marchadier, L. ('04). Étude de la réaction provoquée par un ferment oxydant indirect (anaéroxydase). Compt. rend. Acad. Paris **138**: 1432–1434. 1904.

Brown, W. ('15). Studies in the physiology of parasitism. I. The action of Botrytis cinerea. Ann. Bot. **29**: 313–348. 1915.

Buller, A. H. R. ('05). The destruction of wooden paving blocks by the fungus, Lentinus lepideus Fr. Jour. Econ. Biol. **1**: 1–12. 1905.

———, ('06). The enzymes of Polyporus squamosus Huds. Ann. Bot. **20**: 49–59. 1906.

———, ('06a). The biology of Polyporus squamosus Huds., a timber-destroying fungus. Jour. Econ. Biol. **1**: 101–138. *pl. 5–9.* 1906.

———, ('09). Researches on fungi. pp. 1–287. *pl. 1–5. f. 1–83.* 1909.

Butkewitsch, W. ('03). Umwandlung der Eiweissstoffe durch die niederen Pilze im Zusammenhange mit einigen Bedingungen ihrer Entwickelung. Jahrb. f. wiss. Bot. **38**: 147–240. 1903.

Clark, E. D. ('10). The plant oxidases. pp. 1–111. 1910.

———, ('11). The nature and the function of the plant oxidases. Torreya **11**: 23–31, 55–61, 84–92, 101–110. 1911.

Cooley, J. S. ('14). A study of the physiological relations of Sclerotinia cinerea (Bon.) Schröter. Ann. Mo. Bot. Gard. **1**: 291–326. 1914.

Cousin, H., et Hérissey, H. ('08). Oxydation de l'eugénol par le ferment oxydant des champignons et par le perchlorure de fer; obtention de dehydrodieugénol. Compt. rend. Acad. Paris **146**: 1413–1415. 1908.

Cross, C. F., and Bevan, E. J. ('01). Researches on cellulose, I, 1895–1900. pp. 1–180. 1901.

———, ———, ('06). *Ibid.* II, 1900–1905. pp. 1–184. 1906.

———, ———, ('12). *Ibid.* III, 1905–1910. pp. 1–173. 1912.

Czapek, F. ('99). Ueber die sogenannten Ligninreaction des Holzes. Zeitschr. f. physiol. Chem. **27**: 141–166. 1899.

———, ('99a). Zur Biologie der holzbewohnenden Pilze. Ber. d. deut. bot. Ges. **17**: 166–170. 1899.

———, ('13). Biochemie der Pflanzen. **1**: 1–828. Jena, 1913. [See pp. 682–694.]

Davis, A. R. ('15). Enzyme action in the marine algae. Ann. Mo. Bot. Gard. **2**: 771–836. 1915.

Dean, A. L. ('03). Experimental studies on inulase. Bot. Gaz. **35**: 24–35. 1903.

Delezenne, C., et Mouton, H. ('03). Sur la présence d'une kinase dans quelques champignons Basidiomycetes. Compt. rend. Acad. Paris **136**: 167–169. 1903.

————, ('03a). Sur la présence d'une erepsine dans les champignons Basidiomycetes. *Ibid.* **136**: 633–635. 1903.

Dox, A. W. ('09). The intracellular enzymes of lower fungi, especially those of Penicillium Camemberti. Jour. Biol. Chem. **6**: 461–467. 1909.

————, ('10). The intracellular enzymes of Penicillium and Aspergillus. U. S. Dept. Agr., Bur. An. Ind. Bul. 120: 1–70. 1910.

————, und Neidig, E. ('13). Enzymatische Spaltung von Hippursäure durch Schimmelpilze. Zeitschr. f. physiol. Chem. **85**: 68–71. 1913.

Duggar, B. M. ('05). The principles of mushroom growing and mushroom spawn making. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 85: 1–60. *pl. 1–7.* 1905.

Euler, H. ('06). Zur Kenntnis der Katalase. Beitr. z. chem. Physiol. u. Path. **7**: 1–15. 1906.

————, ('12). General chemistry of the enzymes. pp. 1–323. 1912. [Translation from German by Thos. H. Pope.]

Falck, R. ('02). Die Cultur der Oidien und ihre Ruckfuhrung in die hohere Fruchtform bei den Basidiomyceten. Beitr. z. Biol. d. Pflanzen **8**: 307–346. *pl. 12–17.* 1902.

————, ('09). Die Lenzites-fäule des Coniferholzes. Möller's Hausschwammforschungen. Heft **3**: 1–234. *pl. 1–7. f. 24.* Jena, 1909.

————, ('12). Die Merulius-fäule des Bauholzes. *Ibid.* Heft **6**: 1–405. *pl. 1–17. f. 73.* Jena, 1912.

Ferguson, Margaret C. ('02). A preliminary study of the germination of the spores of Agaricus campestris and other basidiomycetous fungi. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 16: 1–43. *pl. 1–3.* 1902.

Gerber, M. C. ('09). La présure des Basidiomycetes. Compt. rend. Acad. Paris **149**: 944–947. 1909.

Grafe, V. ('04). Untersuchungen über die Holzsubstanz vom chemisch-physiologischen Standpunkte. K. Akad. Wiss., Wien, math.-naturw. Kl., Sitzungsber. **113**: 253–295. 1904.

Green, J. R. ('88). On the germination of the tuber of the Jerusalem artichoke (Helianthus tuberosus). Ann. Bot. **1**: 223–236. 1888.

————, ('93). On vegetable ferments. *Ibid.* **7**: 83–137. 1893.

Hartig, R. ('78). Die Zersetzungscheinungen des Holzes der Nadelholzbäume und der Eiche. pp. 1–151. *pl. 1–21.* Berlin, 1878.

Hawk, P. B. ('12). Practical physiological chemistry. pp. 1–475. 1912.

Helbig, Dr. ('11). Notiz ueber den Cellulosegehalt von Eichenholz, welches durch Thelephora perdix verändert war. Naturwiss. Zeitschr. f. Forst- u. Landw. **9**: 246–250. 1911.

Hjort, J. ('96). Neue eiweissverdauende Enzyme. Centralbl. f. Physiol. **10**: 192–194. 1896.

Humphrey, C. J., and Fleming, Ruth M. ('15). The toxicity to fungi of various oils and salts, particularly those used in wood preservation. U. S. Dept. Agr., Bul. 227: 1–38. *pl. 1–4.* 1915.

Iwanoff, L. ('03). Ueber die fermentative Zersetzung der Thymo-nucleinsäure durch Schimmelpilze. Zeitschr. f. physiol. Chem. **39**: 31–43. 1903.

Jean, F. ('00). Die Bestimmung des Tannins und der Gallussäure. Ann. Chim. anal. appl. **5**: 134–140. 1900. [Chem. Centralbl. **1900**^{1}: 1107–1108. 1900.]

Johnson, J. B. ('93). Timber physics. Investigations on long-leaf pine. 4. Results on mechanical tests. U. S. Dept. Agr., For. Div. Bul. 8: 22–31. *f. 11–16.* 1893.

Jones, L. R. ('05). The cytolytic enzymes produced by Bacillus carotovorus and certain other soft rot bacteria. Centralbl. f. Bakt. II. **14**: 257–272. 1905.

Kellerman, K. F., and McBeth, I. G. ('12). The fermentation of cellulose. *Ibid.* **34**: 485–494. *pl. 1–2.* 1912.

Kikkoji, T. ('07). Ueber das Vorkommen von einem Nucleinsäurespaltenden Fermente in Cortinellus edodes. Zeitschr. f. physiol. Chem. **51**: 201–206. 1907.

Knudson, L. ('13). Tannic acid fermentation. I. Jour. Biol. Chem. **14**: 159–184. *f. 1–2.* 1913.

———, ('13a). *Ibid.* II. Effect of nutrition on the production of the enzyme, tannase. *Ibid.* **14**: 185–202. 1913.

Kohnstamm, P. ('01). Amylolytische, glycosidspaltende, proteolytische und Cellulose lösende Fermente in holzbewohnenden Pilzen. Bot. Centralbl., Beih. **10**: 90–121. 1901.

Kossowicz, A. ('12). Die enzymatische Natur der Harnsäure- und Hippursäure-Garung. Zeitschr. f. Garungsphysiol. **1**: 121–123, 317–319. 1912.

Le Renard, A. ('12). Influence du milieu sur la résistance du Pénicille crustacé aux substances toxiques. Ann. Sci. Nat., Bot. IX. **16**: 277–336. 1912.

Lutz, M. L. ('12). Sur la présence dans le Gyromitra gigas et le Disciotis perlata de tyrosinase et d'un chromogene. Soc. Myc. Fr., Bul. **28**: 136–139. 1912.

Lyman, G. R. ('07). Culture studies on polymorphism of Hymenomycetes. Boston Soc. Nat. Hist., Proc. **33**: 125–209. *pl. 18–26.* 1907.

McBeth, I. G., and Scales, F. M. ('13). The destruction of cellulose by bacteria and filamentous fungi. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 266: 1–52. *pl. 1–4.* 1913.

Mangin, L. ('92). Propriétés et réactions des composés pectiques. Jour. de Bot. **6**: 206–212, 235–244, 363–368. 1892.

———, ('93). *Ibid.* **7**: 37–47, 121–131, 325–343. *pl. 1–2.* 1893.

Miyoshi, M. ('95). Die Durchbohrung von Membranen durch Pilzefäden. Jahrb. f. wiss. Bot. **28**: 269–289. *f. 1–3.* 1895.

Münch, E. ('09). Untersuchungen über Immunität und Krankheitsempfänglichtkeit der Holzpflanzen. Naturwiss. Zeitschr. f. Forst- u. Landw. **7**: 54–75, 87–114, 129–160. *f. 1–5.* 1909.

Newcombe, F. C. ('99). Cellulose-enzymes. Ann. Bot. **13**: 49–81. 1899.

Oppenheimer, C. ('10). Die Fermente und ihre Wirkungen. pp. 1–606. 1910.

Pringsheim, H. ('08). Ueber Pilzdesamidase. Biochem. Zeitschr. **12**: 15–25. 1908.

Reed, H. S. ('13). The enzyme activities involved in certain fruit diseases. Va. Polytec. Inst., Agr. Exp. Sta. Rept. **1911-1912**: 51–77. 1913.

Richards, H. M. ('97). Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Jahrb. f. wiss. Bot. **30:** 665–688. 1897.

Rumbold, C. ('08). Beiträge zur Kenntnis der Biologie holzzerstörender Pilze. Naturwiss. Zeitschr. f. Forst- u. Landw. **6:** 81–140. *pl. 1. f. 1–22.* 1908.

Schellenberg, H. C. ('08). Untersuchungen ueber das Verhalten einige Pilze gegen Hemicellulosen. Flora **98:** 257–308. 1908.

von Schrenk, H. ('00). Two diseases of red cedar caused by Polyporus juniperinus n. sp. and Polyporus carneus Nees. U. S. Dept. Agr., Veg. Physiol. and Path. Bul. 21: 1–22. *pl. 1–7. f. 1–3.* 1900.

————, ('00a). Some diseases of New England conifers. *Ibid.* 25: 1–56. *pl. 1–15. f. 1–3.* 1900.

————, ('00b). A disease of Taxodium distichum known as peckiness, also a similar disease of Libocedrus decurrens known as pin-rot. Mo. Bot. Gard. Rept. **11:** 23–77. *pl. 1–6.* 1900.

————, ('01). A disease of the black locust (Robinia Pseudacacia L.). *Ibid.* **12:** 21–31. *pl. 1–3.* 1901.

————, ('03). A disease of the white ash caused by Polyporus fraxinophilus. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 32: 1–20. *pl. 1–5.* 1903.

————, ('14). Two trunk diseases of the mesquite. Ann. Mo. Bot. Gard. **1:** 243–252. *pl. 6–7.* 1914.

————, ('14a). A trunk disease of the lilac. *Ibid.* 253–262. *pl. 8–9.* 1914.

Schwalbe, C. G. ('11). Die Chemie der Cellulose unter besonderer Berücksichtigung der Textil- und Zellstoffindustrien. Berlin, 1911.

Shaffer, P. A. ('14). On the determination of sugar in blood. Jour. Biol. Chem. **19:** 285–295. 1914.

Shibata, K. ('04). Ueber das Vorkommen von Amide spaltenden Enzymen bei Pilzen. Beitr. z. chem. Physiol. u. Path. **5:** 384–394. 1904.

Singer, M. ('82). Beiträge zur naheren Kenntnis der Holzsubstanz und der verholzten Gewebe. K. Akad. Wiss., Wien, math.-naturw. Kl., Sitzungsber. **86:** 345– 1882. (Cited by Grafe, '04, p. 357.)

Spaulding, P. ('11). The timber rot caused by Lenzites saepiaria. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 214: 1–46. *pl. 1–4. f. 1–3.* 1911.

Vines, S. H. ('03). Proteolytic enzymes in plants. II. Ann. Bot. **17:** 297–316. 1903.

Ward, H. M. ('88). A lily-disease. *Ibid.* **2:** 319–382. *pl. 20–24.* 1888.

————, ('97). On the biology of Stereum hirsutum Fr. Roy. Soc. London, Phil. Trans. **189^{2}:** 123–134. *pl. 17–21.* 1897.

————, ('98). Penicillium as a wood-destroying fungus. Ann. Bot. **12:** 565–566. 1898.

Wehmer, C. ('12). Hausschwammstudien. I. Zur Biologie von Coniophora cerebella A. & Sch. Myc. Centralbl. **1:** 2–10; *Ibid.* III. **2:** 331–340. 1912.

————, ('14). *Ibid.* IV. Versuche ueber die Bedingungen der Holzansteckung und -Zersetzung durch Merulius. *Ibid.* **3:** 321–332. *f. 1; Ibid.* V. **4:** 241–252. *f. 1;* 287–299. *pl. 1–2.* 1914.

————, ('14a). Holzansteckungsversuche mit Coniophora, Trametes, und Polyporus. Ber. d. deut. bot. Ges. **32:** 566–570. 1914.

———, ('14). Die chemische Wirkung des Hausschwamm auf die Holzsubstanz. *Ibid.* **32**: 601–608. 1914.

Weir, J. R. ('14). Notes on wood-destroying fungi which grow on both coniferous and deciduous trees. I. Phytopath. **4**: 271–276. 1914.

Westerdijk, Johanna ('15). Phytopathology in the tropics. Ann. Mo. Bot. Gard. **2**: 307–313. 1915.

Wolf, F. A. ('16). Citrus canker. Jour. Agr. Res. **6**: 69–99. *pl. 8–11. f. 1–8.* 1916.

Wolff, J. ('09). Sur quelques propriétés nouvelles des oxydases de Russula Delica. Compt. rend. Acad. Paris **148**: 500–502. 1909.

EXPLANATION OF PLATE

PLATE 8

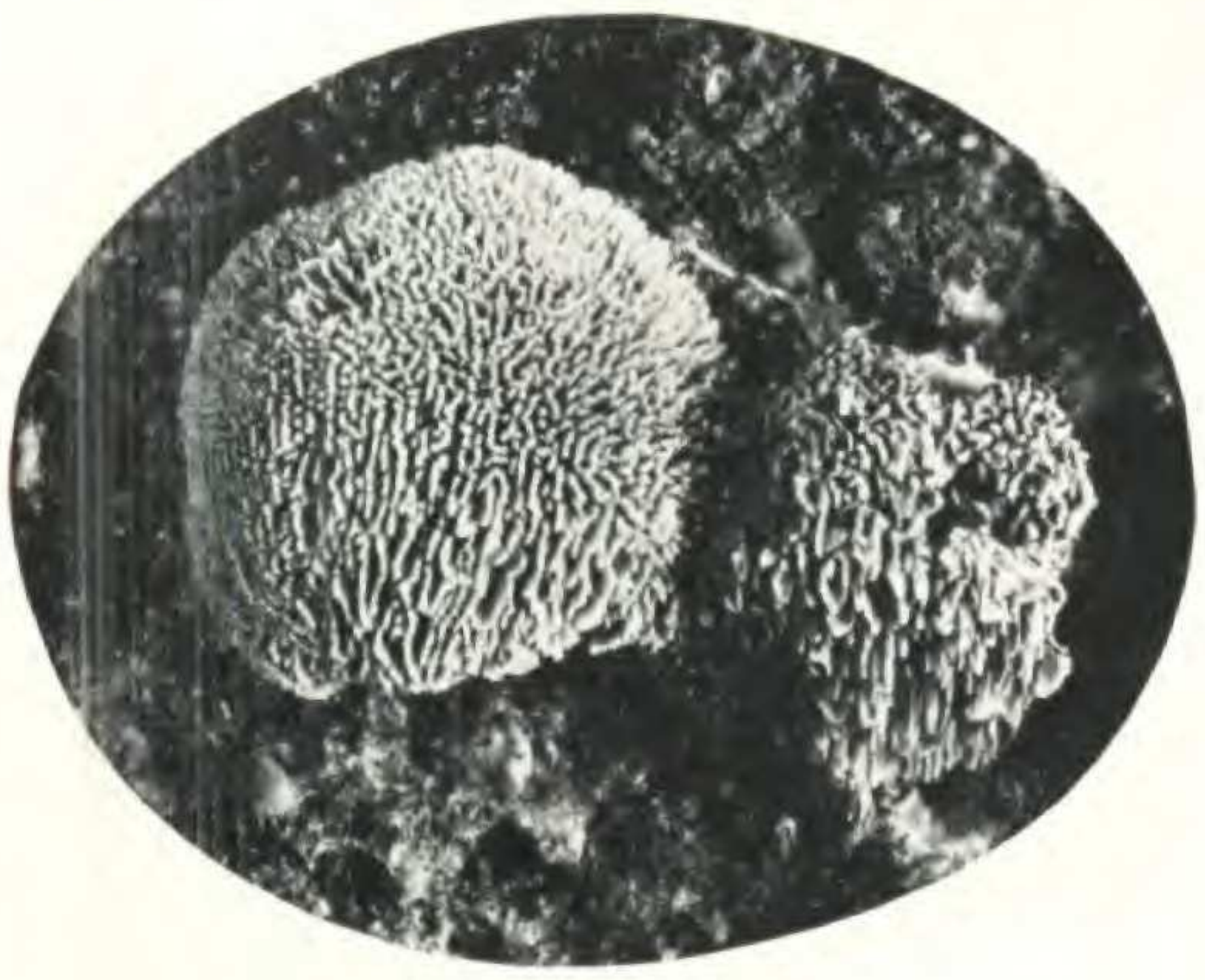
Figs. 1, 3, 4, 6, and 7. Sporophores of *Lenzites saepiaria* growing on sawdust in pure culture, and showing the thelephoroid, daedaloid, and irpiciform characters produced under cultural conditions.

Fig. 2. Sporophores *in situ* on blocks of *Pinus echinata* in pure culture.

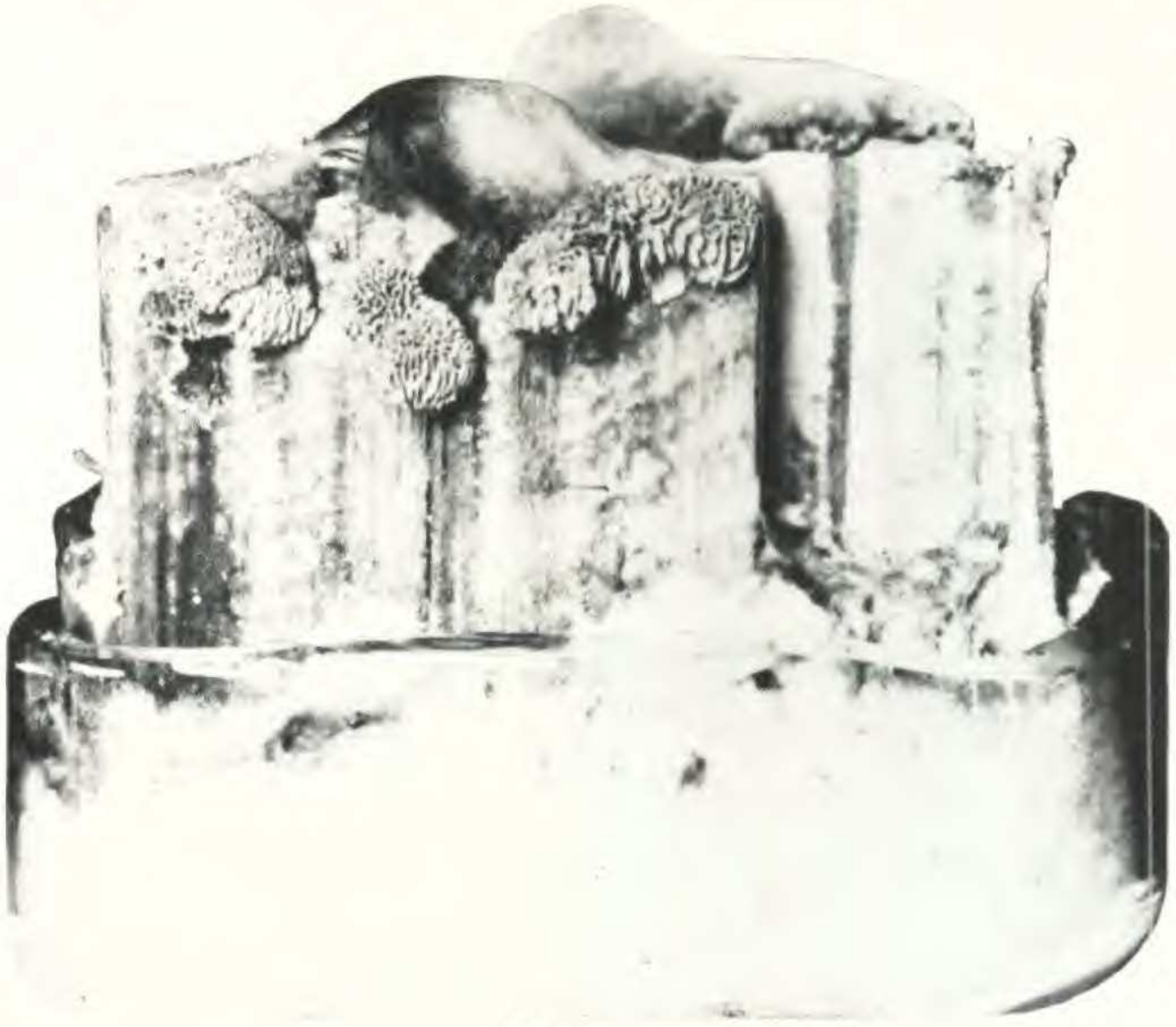
Fig. 5. The lower surface of a sporophore borne on a horizontal surface of a cultural block. The hymenium is borne on typical Lenzites gills.

Figs. 8–12. Samples of pine after one year in culture under favorable moisture conditions, showing the typical internal decay.

Figs. 13–17. Samples of pine showing the superficial "scorching" after one year in culture, during which time the blocks were saturated with water.



1



2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

ZELLER—LENZITES SAEPIARIA

EXPLANATION OF PLATE

PLATE 9

To illustrate the growth of *Lenzites saepiaria* on resin agar plates in Petri dishes of uniform size in 14 days.

Fig. 1. Control plate showing the growth on a Thaxter's glucose-potato-hard agar containing no resin.

Figs. 2–21. Showing the growth on 5–100 per cent resin agar, respectively (each plate increased by 5 per cent resin by weight), where Thaxter's glucose-potato-hard agar was used as a basis.